COMPLEX REGIONAL PAIN SYNDROME

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COMPLEX REGIONAL PAIN SYNDROME
formerly called reflex sympathetic dystrophy syndrome
treatment with ketanserin and carnitine

GECOMPLICEERD – LOCAAL – PIJN – SYNDROOM
voorheen genoemd sympathische reflex dystrofie
de behandeling met ketanserin en carnitine

PROEFSCHRIFT

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Chapter 1 SCOPE OF THE THESIS

1.1 Aim of the work

Inhibition of the microcirculation causes ischemia and pain. Ischemia gives rise to pain and vice versa. It is seldom clear which phenomenon is first. Ischemia is the result of vasoconstriction which is on its turn mainly caused by the action of the neurotransmitter serotonin on the sympathetic nerve endings. Ischemia inhibits the process of oxidative phosphorylation. The cellular structure of the micro vessels implies that the endothelial and smooth muscle cells are the first to deal with this metabolic change. Ischemia causes a release of transmitters, hormones and cytokines in an attempt to restore energy metabolism. Extracellular ATP molecules play an important role in these processes and may bind to specific receptors on the sympathetic nerve endings.

In regional peripheral areas of susceptible individuals, ischemia plus pain may cause the Complex Regional Pain Syndrome (CRPS).

We studied the relationship between plasma carnitine levels and age in both healthy female volunteers and CRPS patients. In addition, the effect of ketanserin on the plasma carnitine level was investigated.

The main purpose of this work is to show that these conditions of diminished local flow and derangements of the aerobic metabolism and the pain in CRPS patients could be alleviated and even cured by relieving the vasoconstriction with the 5-HT_{2A} receptor antagonist ketanserin and by correcting the metabolic changes with carnitine.

Chapter 2 INTRODUCTION TO CRPS

2.1 A disease with an ever-changing name

Throughout history there have been reports about long-lasting complaints by a complex of symptoms after healing of the primary trauma, wound or fracture. These complaints do not necessarily have a clear relationship with the original trauma.

One of the earliest descriptions is from Ambrois Paré (1510–1590). As the physician of King Henry II and Henry III, he was asked to treat the arm of a man who later became King Charles IX. The man was wounded in the arm by a lancet; after the wound was healed he experienced a burning pain in his arm associated with contracture of the muscles.

In 1864 Mitchell and colleagues reported a long-lasting pain syndrome in a patient following gunshot wounds involving nerve injury. The symptomatology described by these authors, in which the symptoms persisted after specific peripheral nerve injury, was called "causalgia", from the Greek words "kausis" meaning heat, and "algos" meaning pain.

These reports discussed the external wounds of patients as well as the difficulty of understanding the relationship between the complaints and healing of the wounds. Volkman (1882) was the first to report the occurrence of rarefaction of bone in some cases of posttraumatic healing. Later, with the introduction of new radiological techniques to study e.g. bone fractures, Destot (1898) described singular osteoporosis following a persistently painful ankle sprain.

Sudeck in 1900 was the first clinician to report on a complex of symptoms that can persist following trauma of a limb. In his view this involved a syndrome which was characterized by burning pain, edema and limitation of motion in the affected limb. These symptoms were already associated with local circulatory changes, trophic changes in the tissues, and patchy demineralisation of bone. Since that time, this complex of symptoms has been called "Sudeck's Atrophy". But in the literature, numerous syndromes with a similar complex of symptoms have been described. They all have in common lasting pain, local circulatory changes and dystrophic or atrophic changes in the affected limb.

Table I shows the various names used in the literature to describe this syndrome. The clinicians attempted to define the syndrome according to the symptoms they observed.

Table I. Various names used to describe the syndrome.

-Sudeck's atrophy (Sudeck 1900)
-Acute atrophy of bone (Sudeck 1938)
-Peripheral trophoneurosis (zur Verth 1929)
-Traumatic angiospasm (Morton and Scott 1931)
-Posttraumatic osteoporosis (Fontaine and Herrman 1933)
-Traumatic vasospasm (Lehman 1934)
-Trophic edema (Homans 1939)
-Chronic segmental angiospasm (Homans 1940)
-Posttraumatic dystrophy (Miller and de Takats 1942)
-Post-infarctional sclerodactyla (Johnson 1943)

-Shoulder-hand syndrome (Steinbrocker 1947)
-Posttraumatic neurodystrophy (Glick and Helal 1976)
-Algoneurodystrophy (Gobelet 1984)

In several patients a nerve was also damaged; this led to the name causalgia (Mitchell et al. 1864) or minor causalgia (Homans 1940). The connection between the traumatic event and the disproportionate reaction led to the assumption that some type of reflex could be involved; this assumption led to the use of additional names (Table II).

Table II. Names reflecting the involvement of a reflex mechanism.

-Reflex arterial spasm (Homans 1939)
-Reflex dystrophy of the extremities (Homans 1940)
-Reflex nervous dystrophy (de Takats 1943)
-Reflex neurovascular dystrophy (Steinbrocker 1947)

The first clinical evidence that the sympathetic nervous system was involved in the pathogenesis of this symptom complex, associated with a reflex mechanism, was published by Spurling in 1930. He reported a patient with causalgia which he treated by surgical interruption of the cervico-thoracic chain. In 1946 Evans was the first to combine in one term the three elements thought to be involved in this peculiar reaction of a limb after a trauma. Dystrophic changes, possibly evoked by a reflex mechanism involving the sympathetic nerves. This led to the name reflex sympathetic dystrophy. Several authors later incorporated the sympathetic nervous system in the name they used when reporting on this complex of symptoms (Table III).

Table III. Names reflecting the involvement of the sympathetic nervous system.

-Reflex sympathetic dystrophy (Evans 1946)
-Posttraumatic sympathetic dystrophy syndrome (Drucker et al. 1959)
-Sympathetically maintained pain (Roberts 1986)
-Reflex sympathetic dystrophy syndrome (Davidoff et al. 1988)

Until recently, all the mentioned syndromes were combined under the name Reflex Sympathetic Dystrophy Syndrome (RSDS). Despite the fact there was consensus about the name, there was no consensus about the definition of the syndrome. Even in recent years the discussion continues about what the exact description of RSDS should be. In 1986 the Taxonomy Subcommittee of the International Association for the Study of Pain (IASP) formulated an as accurate as possible definition of RSDS. This first definition stated: "Continuous pain in a portion of an extremity after trauma, which may include fracture but does not involve a major nerve, associated with sympathetic hyperactivity". However, by 1990 Stanton-Hicks et al. presented a proposal to the Taxonomy Subcommittee to change this definition to one that was clinically more precise, namely: "A syndrome of continuous diffuse limb pain, often burning in nature, and usually consequent to injury or a noxious stimulus, or disuse, presenting with variable sensory, motor, autonomic and trophic changes: causalgia represents a specific presentation of RSDS associated with peripheral nerve injury". The most striking difference between these two definitions is that the definition by Stanton-Hicks et al. also includes nerve injury. Since this difference is, in fact, only a matter of dividing classes, for clinical

use an appropriate description of the condition is a chronic pain syndrome in which the autonomic sympathetic nervous system is involved. This pain syndrome is located at the extremities and is called "reflex sympathetic dystrophy syndrome", RSDS; this was the meaning in 1990.

But in 1995 Stanton-Hicks, Jänig, Hassenbusch, Haddox, Boas and Wilson presented yet another proposal: this was a revised taxonomic system for disorders previously called reflex sympathetic dystrophy and causalgia. The taxonomy system was the result of a special consensus conference on the topic, and is based on patient's history, presenting symptoms, and findings at the time of diagnosis. The disorders were grouped under the term "complex regional pain syndrome", CRPS. This overall term, CRPS, requires the presence of regional pain and sensory changes following a noxious event. Further, the pain is associated with findings such as abnormal skin colour, temperature change, abnormal sudomotor activity, or edema. The combination of these findings exceeds their expected magnitude in response to known physical damage during and following the inciting event. Two types of CRPS have been recognized: type I occurs without a definable nerve lesion; and type II, formerly called causalgia, refers to patients with a definable nerve lesion. The term sympathetically maintained pain was also evaluated and considered to be a variable phenomenon associated with a variety of disorders, including CRPS types I and II. These revised categories were included in the second edition of the IASP Classification of Chronic Pain Syndromes.

2.2 Clinical symptoms

The clinical picture is characterized by the appearance of a complex of symptoms which is extensively described in the recommendations and guidelines of the Taxonomy Subcommittee and by Stanton-Hicks et al. (1995). One of the main problems of CRPS is, that the complex of symptoms is not consistent with regard to time and composition. One consistent central symptom is, the continuous pain in the affected limb. The pain is of a burning nature, is intensified by movement and is accompanied by local vasoconstriction or sometimes, especially in the early stages, by vasodilatation, leading to changes in the colour of the skin. After some time, weeks or months, trophic changes may occur, such as atrophy of the skin and coarsening of the skin and nails. Deeper structures, e.g. muscles and joints, may become stiff and in some patients the hair may grow quickly or fall out. The symptoms and changes spread independently of both the source and site of the precipitating event.

According to Stanton-Hicks et al. (1995), the symptoms can be divided in five groups:

1. Sensory abnormalities: burning pain at rest, worsening by movement, hypo- or hyperesthesia, allodynia to cold and mechanical stimulation.
2. Motor dysfunction: muscle weakness, tremor, joint stiffness.
3. Autonomic dysregulation: alterations in blood flow, hyperhidrosis, edema.
4. Trophic changes: skin atrophy, coarsening of skin and nails, rapid growth or loss of hair.

5. Psychologic reactive disturbances: anxiety, depression, hopelessness (as in other chronic pain patients).

According to the Taxonomy Subcommittee, the CRPS syndrome often has a preceding trauma which can be very mild or even not mentioned. The preceding event can be a herniated intervertebral disc, spinal anesthesia, poliomyelitis, ileo-femoral thrombosis or cardiac infarction. But in the majority of CRPS patients one can find sprain, mild frostbite, burns, partial nerve injuries, venous thrombosis, low-grade infections, and fractures of wrist and ankles. There may be a lapse of several weeks between the possible cause and the manifestation of the syndrome. The natural course of CRPS is rather unpredictable. CRPS immediately after a mild trauma can be of a mild form and sometimes spontaneous remission may occur within a few weeks. On the other hand, long lasting types of CRPS may persist indefinitely, often involving three stages (see Table IV), and which may eventually lead to severe disability. The severity of the original injury does not always determine the course of the disease. In fact, severe trauma causing fractures of long bones and transections of nerves or blood vessels is seldom followed by CRPS. In patients with symptoms which might suggest CRPS, one should always be aware of unrecognized local pathology such as fractures, strain or sprain, posttraumatic vasospasm or thrombosis, or neuroma.

2.3 Diagnostic tests

Due to the lack of a clear definition of CRPS, and because CRPS often presents differently at various stages, it is difficult to clearly define the diagnostic criteria. Besides the clinical presentation of various symptoms, there are clinical tests which may aid the diagnostic process and establish the stage of CRPS.

2.3.1 Skin temperature measurements

Bilateral symmetric measurements of skin temperature by means of surface thermistors may reveal consistent differences. Thermography allows to image the thermal condition of the skin. Investigation by means of thermistors or thermography is preferable to subjective evaluation and enables to obtain a clear image of abnormal skin temperature. Thermography also allows easy follow-up of the effectiveness of therapy (Hematsu 1983, Ecker 1984).

2.3.2 Sensory and motor tests

Pin pricks, light touch, mechanical stimulation and touching with cold objects can be used to detect abnormal thresholds in cases of hypo- or hyperesthesia and allodynia. Both active and passive movements can be used to determine reduced measures of strength, weakness, tremor or stiffness.

2.3.3 Radiological findings

Radiological findings in bone are not decisive in the diagnosis of CRPS and they may not reflect the severity of the condition. Severe CRPS may be encountered without radiological symptoms. CRPS of only two months duration may show radiological

symptoms, whereas CRPS existing for two years or more could have no radiological pathology.

In patients with fractures, radiology may reveal normal healing of the fracture despite CRPS-like complaints from the patients, e.g. pain, edema and hyperhidrosis. It must be noted, however, that radiological findings may not appear until there is bone demineralisation of about 30–40%. The first radiological signs of CRPS include symptoms such as subchondral bone resorption, together with spots of bone resorption in the epiphyseal area. In the next phase, the cortex of the epiphysis becomes thin. Demineralisation of the bone can be very progressive. At a later stage, the radiological findings may appear almost the same as in osteoporosis. Bone demineralisation can lead to extreme changes in the trabecular structure of the bone.

2.3.4 Scintigraphic findings

Using the isotope ^{99}Tc enables to acquire information about the blood pool, the relative vascularisation of the soft tissues, and the metabolism of the bone. Several researchers have reported their experiences with three-phase bone scanning in CRPS (Kozin et al. 1981a, b, Holder et al. 1984, Demangeat 1987) demonstrating that the sensitivity and specificity of scintigraphy is better than that of radiology. Some even claim that, using different parameters, it is possible to identify three stages of CRPS. On the other hand, Allen et al. (1999) questioned the value of the three-phase bone scan. In a retrospective study (n=51) they found in only approximately one half (53%) of the patients an interpretation of the radiologist, that was "consistent with the diagnosis CRPS".

2.3.5. Urine biochemistry

According to Doury et al. (1981) and Shiano et al. (1981), hydroxyproline in urine is increased in an early stage of CRPS. Others (Gobelet 1984) pointed out that this finding was positive in 39% of patients with CRPS, and that in 47% the enzyme alkaline phosphatase was abnormally high in the early phase of stage three of CRPS (see 2.4.2.).

2.3.6. Phospho-nuclear magnetic resonance spectroscopy

Radda (1986) reported that in normal human muscle there is a ratio between inorganic phosphate and phosphocreatine of 0.13 ± 0.02. In patients with CRPS, and especially in an advanced stage, Goris et al. (1987) reported a ratio of 0.15 to 0.25. Because phosphocreatine is synthesised from creatine and ATP, this finding suggests the possibility of a disturbed oxidative phosphorylation process.

2.4 Natural course of CRPS

The clinical picture of patients referred to pain management departments shows a broad variety of symptoms. Well-trained clinicians are able to classify the symptoms based on the time course of the disease and the severity of the complaints.

2.4.1 Course of CRPS according to Steinbrocker, with a happy ending

Steinbrocker (1947) was the first to describe a classification in which stage three was the desired natural rehabilitation without complaints.

Stage I was clinically characterized by pain, raised skin temperature, hyperalgesia, hyperesthesia and edema, due to vasodilatation distal to the original trauma.
Stage II was characterized by sweating, trophic disturbance and roentgenographic changes (spotty osteoporosis) caused by vasoconstriction.
Stage III was the convalescent phase with normalisation of the pathological parameters.

2.4.2 Course of CRPS according to Bonica, with a realistic outcome

Bonica (1953) described the realistic stage III to which unfortunately many CRPS patients are destined. The progression to stage III generally occurs because a spontaneous remission of stage II, or improvement without treatment, is very rare. Despite the unfortunate succession of symptoms, CRPS always has been, and probably will remain, a dynamic syndrome which does not remain stable in most patients for any length of time. Therefore, the delineation between the stages is not always clear. With these restrictions in mind, Bonica could nevertheless distinguish three stages in the following way (Table IV):

Table IV. Major stages of CRPS as defined by Bonica (1953).

	Pain	**Trophic changes**	**Autonomic instabilities**	**Sensory abnormalities**	**Bony changes**
Stage I	Burning pain at rest, aggravated by movement	Local muscle spasm	Warm, red, dry or cool, pale, local edema	Hyperesthesia	Some spotty osteoporosis, periarticular
Stage II	Pain may increase, decrease or stabilise and spread to proximal	Local joint becomes stiff	Skin; cold, pale, cyanotic, edema, spreading	Hyperesthesia, paraesthesia, allodynia	Osteoporosis progresses to diffuse
Stage III	Pain is variable, often increased	Severe trophic changes, immobilisation of the limb, atrophy and contracture of muscles	Skin smooth, cold, no edema	Spread of allodynia and and dysesthesia	Diffuse osteoporosis, decalcification of small bones

Stage I

In the first stage the extremity is warm, showing edema of subcutaneous tissues and joint capsule, the muscles are hypertonic with a tendency to spasm. The pain is restricted to the site of injury, and the tendency of the disease to spread is not yet evident. Movement worsens the pain and hyperesthesia is frequently present. Osteoporosis may occur after 4 to 8 weeks of continuous collateral hyperemia. Initially, a spotty osteoporosis appears in the junctura-articular bone of the affected limb. This begins in the metacarpal-phalangeal or metatarsal-phalangeal joints.

Stage II

In the second stage the edema spreads. Because of the persisting synovial edema, the joints become stiff. Regional changes with muscle stiffness, soreness and myofascial

irritation spread from distal to the girdle. The dystrophic limb is cool and pale, or cyanotic and sweaty. Pain generally increases, but may decrease with strict immobilisation. The sympathetic hyperactivity can cause the hair and nails to become thickened and coarse. The osteoporosis is progressive and spreads to become diffuse and involving more joints. Hyperesthesia may be more prominent and in severe cases dysesthesia and allodynia may occur.

Stage III
Finally, in the third stage there is a diffuse decalcification of the small bones, pain is variable but mostly increased. There are severe trophic changes. The allodynia and dysesthesia spread. The extremity is totally immobile, there is progressive ankylosis of the joints and contracture of the muscles. The skin is smooth, thin and shiny; the skin temperature is decreased and there is no edema.

2.5 Symptoms used in the current work

Not every case of CRPS will follow the three main stages indicated. CRPS sometimes exhibits a mild form of complaints which subsides spontaneously within a few weeks or rapidly responds to treatment. Sometimes CRPS has a self-limiting course that may heal spontaneously within one year. In contrast, there are severe cases which progress to total disability with continuous pain, increasing contracture of tendons and joints, and marked decalcification of bones. In spite of many therapeutic measures, stiffness, deformity, and contracture of the joints may persist. Thus the course of CRPS in these patients can also be very severe with spreading neuralgia, spreading osteoporosis and mental fixation on an intractable lesion. This can lead to situations in which the patient may request amputation, and even threaten to commit suicide. Taking into account the dynamic picture of CRPS, it is extremely important to establish a consensus about the diagnosis of CRPS. However, due to the absence of firmly established clinical criteria for CRPS the diagnosis may vary between physicians, therefore the first step must be to arrive at some agreement on the diagnostic criteria. The IASP Taxonomy Subcommittee has addressed this difficult problem and has published general recommendations and guidelines for the diagnosis of CRPS. These guides are very broad and encompass all the possible clinical manifestations, as well as the possible clinical and scientific investigations. Anamnestic information and the clinical picture are decisive in the recognition of CRPS. According to the guidelines of the pain chapter of the Dutch Society of Anesthesiologists (1996) the clinician has to accept the diagnosis of CRPS when three of the following five symptoms are present: abnormal skin temperature, abnormal skin colour, edema, inexplicable progression of pain by movement, and inexplicable impaired mobility. I have compiled a list of symptoms related to the diagnosis of CRPS (Table V; Moesker 1992) with more attention to the differentiation in the pain symptomatology.

In this view, the first symptom is that of persistent, burning pain at rest. The second symptom is exacerbation of pain by movement, cutaneous stimulation or stress. Besides these two main symptoms of pain, the associated symptoms of the circulation and the autonomic nervous system are included, such as: impaired mobility, edema, hyperhidrosis abnormal skin temperature and hyperpathia and/or allodynia. In individual patients

with CRPS there will always be a complex of symptoms which are present to some degree. But, these symptoms can vary over time. These variations are not only related to the severity of symptoms, but the associated symptoms may not be present in every patient and may not occur at the same time. Therefore, we have accepted the diagnosis CRPS when at least four of the seven symptoms listed in Table V are present.

Bruehl et al. (1999) reported on the external validation of IASP diagnostic criteria for CRPS and proposed diagnostic criteria for research purposes. Their recommendations confirmed the criteria we chose for our investigations. The seven symptoms used in our studies, i.e. persistent pain at rest, increasing pain during exercise, impaired mobility, edema, hyperhidrosis, abnormal skin temperature, hyperpathia/allodynia (of which a minimum of 4 had to be present), were in the four categories they mentioned. They proposed that at least one symptom in each or the four following categories must be present: sensory, vasomotor, sudomotor/edema, and motor/trophic.

Table V. Symptoms related to the diagnosis of CRPS used throughout this work.

1. Persistent pain at rest
2. Increasing pain during exercise
3. Impaired mobility
4. Edema
5. Hyperhidrosis
6. Abnormal skin temperature
7. Hyperpathia and/or allodynia

2.6 Incidence of CRPS

Many authors (Gurd 1938, Jordan 1940, Herrman et al. 1942, Scheibe et al. 1954), have claimed that CRPS is the most common cause of prolonged disablement after injury. Bonica (1953) reported that a clinical diagnosis of CRPS in patients referred to his hospital could be made in about 5% of all trauma cases. Plewes (1956) stated that CRPS is present in about one in 2000 accidents of all kinds. That CRPS tends to favour females, was confirmed by Abram (1976) who reported an incidence of 61%. In our first patient study population of 1984–1988 (n=45) 67% were females (Moesker 1991).

2.7 Incidence of causalgia

When discussing the incidence of CRPS it must be taken into account that the incidence and the epidemiology of causalgia and CRPS are different. Causalgia, as a complex of symptoms after nerve injury, is often described in trauma patients injured during war. In the latter case, male patients are in the majority. Because of the special etiology, the incidence of CRPS after nerve injury, called causalgia, must be distinguished from CRPS representing a complex of symptoms after a host of other known and unknown precipitating injuries and conditions. In cases of causalgia Ulmer et al. (1946) and Omer et al. (1971) also reported an incidence of 5% (n=1400) among nerve injured patients. Bonica (1979), reviewing a number of published series stated that the incidence of causalgia was 2.5–5%, whereas Jebara et al. (1987) reported 5.8% (n=345). Therefore,

one might say that the incidence of causalgia is about 5% in nerve injured trauma patients.

2.8 Preferential age of CRPS

The preferential age of CRPS is reported to be between 40 and 60 years (Pak et al. 1970, Kleinert et al. 1974). In this patients the mean age was 45.3 years (n=48). But CRPS can also occur in childhood; Blau (1984) reported incidental cases. In 1978 Bernstein et al. reported a series of 10 patients with CRPS aged 8 to 17 years and Goldsmith et al. (1986) reported a series of 15 patients aged 9 to 18 years. In our pain clinic, we have treated a young boy aged 8 years. After six weeks immobilisation due to a tibia fracture the boy could not progress to revalidation because of severe CRPS complaints. He had an extremely cold skin, as well as hyperhidrosis, hyperesthesia, and pain at rest which increased with movement; the child could not even touch the floor with his foot. We were able to treat this CRPS successfully with 100% recovery. To our knowledge, the youngest persons with CRPS reported in the literature were aged three years (Kozin et al. 1977, Richlin et al. 1978).

2.9 Primary causal events

There are no data available on the main causes of CRPS. While Pak et al. (1970) and Kleinert et al. (1974) reported blunt trauma as the leading cause of CRPS, Carron et al. (1974) stated that fractures are the leading cause of CRPS. In this series of patients 44% had fractures, 38% had blunt trauma and 18% had various other minor traumas as the starting point of CRPS. The severity of the original injury does not appear to determine the course of the CRPS.

2.10 Personality characteristics

After studying CRPS patients, clinicians may get the impression that they are treating patients with an unstable and hyper-reactive autonomic nervous system. Whether this is the cause or the result of the prolonged disablement is hard to determine. Discussions about CRPS often contain terms such as: "a CRPS personality". However, studies by Wilson (1981) and Haddox et al. (1983) did not show any psychological predisposing factor. Nevertheless, for decades, discussion has continued about the existence of the so-called "Sudeck personality" (Orbach 1934, Takats de 1943, Nippert 1955). CRPS patients were characterized as anxious, tense, inactive and hypersensitive. Pollack et al. (1980) even defined two personality types. Studies by Zachariae (1964) showed that patients at risk for CRPS are characterized by aggressive inhibition, lack of self-assurance, self-absorption, self-pity and hysterical personality traits. Most of the investigators tend to refer to the Sudeck personality as being of the narcissistic type. Because neither the degree of CRPS nor its clinical course has a linear relationship with the degree or severity of the causing trauma, there appears to be a psychodynamic factor which influences both

the degree of CRPS and its course. In our pain clinic we have observed extreme psychopathological circumstances in some CRPS patients, and in their behaviour towards their affected limb.

One extreme example was a very sophisticated, well-dressed lady "carrying" her CRPS arm on a beautiful hand-made pillow. She presented her arm as though it was separated from the rest of her body. This type of behaviour, to a lesser extent, is common among many CRPS patients. They appear to delete the picture of the affected limb out of their own body-map. In our opinion, in the therapeutic program of CRPS there is an essential need for psychological help to enable the integration and use of the affected limb as an integral part of the patient's body.

Chapter 3 PATHOPHYSIOLOGY OF CRPS

3.1 Main events

CRPS is mainly generated by mechanical trauma (Livingston 1943, Sunderland 1978, Schott 1986), excitation of spinal visceral afferent neurons (Sweet 1968, Doury et al. 1981, Kozin 1981a), and lesions in the central nervous system (Moskowitz et al. 1958, Kozin et al. 1981b, Wainapel 1984, Schott 1986, Lücking et al. 1988). The relationship between these neural and non-neural factors is not clearly understood. It is, however, generally agreed that the sympathetic nervous system is in some way involved in the generation of the CRPS. An important reason for this assumption, is the experience that temporary or permanent blockade of sympathetic activity to the affected extremity often relieves or abolishes the pain. This does not indicate in which way the sympathetic nervous system is involved in the generation of CRPS. These responses to sympathetic blockade have led to the assumption and use of the term of sympathetically maintained pain: for decades, the most tenable hypothesis of what invoked a widespread disturbance of centrally-mediated autonomic regulation (Drucker et al. 1959, Schutzer et al. 1984).

3.2 Hypotheses on the pathophysiology of CRPS

Many researchers (Mitchell et al. 1864, Lewis 1936, Leriche 1939, De Takats 1941, Devor 1983, Jänig et al. 1984, 1985, 1992), focussed primarily on posttraumatic changes in peripheral tissues as a source of abnormal activity in afferent nociceptor neurons. Others (Bonica 1953, Devor 1983, Burchiel 1984) have assumed that the higher activity on these nociceptors is responsible for the continuous pain in CRPS. They assumed that peripheral nerve damage stimulates the nociceptors. Secondarily, through the spinal cord there will be an activation of the sympathetic efferent neurons, which by means of vasoconstriction leads to peripheral ischemia which again stimulates nociceptors. This results in a vicious circle in which the disease state may become aggravated.

Roberts (1986) presented the hypothesis that CRPS is mediated by activity of low-threshold myelinated mechanoreceptors. This activity of afferent neurons produces a sensitization of wide dynamic range neurons in the spinal cord. This results in activation of sympathetic afferent neurons which, in turn, stimulates the mechanoreceptors, and again creates a vicious circle.

Neurophysiologically, different models for CRPS have been proposed which have in common that, irrespective of the way the vicious circle is formed, it generates and maintains the CRPS. When considering in more detail the possible mechanisms in the peripheral tissue in which the CRPS is generated, an interesting hypothesis was presented by Livingston (1943). The essence of his hypothesis is the assumption that pain sensation in CRPS is the result of the nociceptor activity secondary to dysregulation of peripheral blood flow. These changes are induced by a dysfunction of sympathetic vasoconstrictor nerves supplying the skin vessels, resulting in superficial pain and constriction of the deep

tissue vessels and the bone vessels, causing deep pain and osteoporosis. Blumberg et al. (1989) described in detail the likely pathophysiological change in the peripheral blood flow that is essential for CRPS. The excitation of peripheral sympathetic innervation has consequences for the peripheral blood flow. The cause of the dysregulation of the peripheral blood flow is an imbalance between the pattern or activity (tone) of the vasoconstrictor neurons supplying arteries, arterial vasoconstrictor tone and those of the veins, venous vasoconstrictor tone. When the venous vasoconstrictor tone is higher than the arterial tone, venous return is impaired in the affected regions, capillary filtration pressure increases and edema results. This leads to higher interstitial pressure which may excite peripheral nociceptors. Alternatively or additionally, increased filtration from blood vessels may induce disturbances of the micro milieu which "chemically" excite nociceptors. Excitation of nociceptors then (via a reflex mechanism) maintains the disturbance of vasocontrictor outflow. Again, a vicious circle is in operation.

An overview of the pathophysiology of CRPS was given by Raj (1998). His view is that should any proposed explanation for the pathophysiology of CRPS be able to explain the character of the pain, it should address the relief of pain by sympathetic block in the early phase of the disease, and not in the later phase. According to Raj, CRPS may be thought of as a prolongation of the normal sympathetic response to injury. A sympathetic reflex arc is the normal response to any traumatic injury. Painful afferent impulses from the periphery, travel along A-delta and C fibres through the general nerves to the spinal cord. Then the impulses go through the dorsal roots and synapse in the dorsal horn with interneurons carrying the impulses to:

- ascending tracts, where they are then projected further to the thalamus and finally to the somatosensory cortex,
- the anterior horn, where a motor reflex may be initiated by efferent motor fibers causing muscle contraction,
- the intermediolateral cell column, where the painful message is relayed to the sympathetic nerve cell bodies.

A sympathetic reflex is activated by efferent sympathetic impulses sent out of the spinal cord through the ventral roots to a ramus communicans albus and then into the sympathetic chain to synapse in a sympathetic ganglion. The postganglionic sympathetic fibre leaves the ganglia by way of the ramus communicans griseus, where it travels with the peripheral nerve to the extremity, producing vasoconstriction. This is a reflex, which normally gives way to vasodilatation as part of the orderly progression toward healing. If this sympathetic reflex arc does not shut down but continues to function and accelerate, a sympathetic hyperdynamic state ensues. This results in increased vasoconstriction and tissue ischemia, causing more pain and thus increasing the large number of afferent pain impulses travelling to the spinal cord and reactivating the sympathetic reflex. This sympathetic efferent stimulation enhances the sensitivity of the nociceptor by causing vasoconstriction and ischemia, changes in vascular permeability, and smooth muscle contraction around the nociceptor; sensitivity is also enhanced by the direct action of locally released substances, including norepinephrine, substance P, prostaglandin and bradykinin. According to this vision we can recognise vasoconstriction, ischemia, and pain from a neuropathic origin as the central phenomena of CRPS.

3.3. Definition and classification of neuropathic pain

An overview concerning neuropathic pain was published by Novelli and Trovati in 1998. Pain is defined as "neuropathic" when it originates from lesions of the peripheral or central nervous system. These lesions are the starting point of anomalous and/or ectopic neural discharges, able to generate pain. If lasting long enough, such pathologic neural discharges can also induce histological, functional and biochemical changes of the nervous pathways along which they are conducted, because of the plasticity of the nervous system. These changes often survive the recovery of the lesion, and the pain they generate persists. Many conditions can generate this "pathologic pain" (Coderre et al. 1993).

3.3.1 A classification of neuropathic pain presented by Fields, 1991

1) Painful mononeuropathies with post-traumatic neuromas, entrapment of nerves, plexuses and spinal roots, neuritis of different etiologies, idiopathic forms such as tic douloureux.
2) Painful ischemic, metabolic, infective, neoplastic, etc. polyneuropathies.
3) Deafferentiation pain with surgical or traumatic interruptions of nerves, plexus or roots, post-herpetic neuralgia, phantom pain, lesions of the central nervous system (central pain) caused by tumors, central vascular diseases, traumas etc. located in every part of the CNS from the spinal cord to the neocortex.
4) CRPS.

3.3.2 Clinical features of neuropathic pain

The clinical features of all forms of this type of pain are:

1) Allodynia occurring in some areas of the body, not necessarily the lesioned area (as indicated by scar or other signs of tissue damage). Slight innocuous stimuli induce pain.
2) Hyperalgesia. Slight stimuli, painful in nature, induce an exaggerated pain compared to the stimulus. This phenomenon can be observed in every part of the body.
3) Hyperpathia. Sensation of intense pain with very unpleasant subjective feature, poorly located, hard to describe but with a constant explosive characteristic, accompanied by well-defined behaviour (movement of the stimulated part of the body, facial grimace, vocalization, etc.). Another important characteristic is prolonged and very intense spatial and temporal summation of painful stimuli. Repeated slightly painful and even non-painful stimuli induce a strong pain after a period of latency, which persists for a long time after the stimuli has stopped (temporal summation). This "after pain" can be induced even by only one stimulus.
4) Referred pain. Pain spreads from the lesion and can be felt also in areas of the body distant from it.

These subjective pains are usually very intense and burning in quality, with stabbing or shock-like bursts. These types of pain, if not properly treated, worsen over time as often seen in CRPS. The neuropathic pain types allodynia and hyperpathia are usually expressed in CRPS.

3.3.3 Pathophysiology of neuropathic pain

The pathophysiology of neuropathic pain is still debated and many theories have been proposed and discussed. The following list is a summary based on neurophysiological theories.

A) Peripheral mechanisms. At a peripheral level, the following mechanisms generating neuropathic pain are proposed:

-ephaptic crosstalk (pathological connections between sympathetic and nociceptive nerves) (Granit et al. 1945, Seltzer et al. 1979, Mayer et al. 1986)
-spontaneous discharge from a neuroma (Wall et al. 1974)
-ectopic firing from the ganglia of dorsal roots (Kirk 1974)
-ectopic firing from lesions or demyelinated areas of peripheral nerves (Calvin et al. 1977)
-depolarizing after-potentials (Raymond 1979)
-generation of after-discharges, (Burchiel 1984)
-reflex spikes (Calvin et al. 1982)
-sympathetic involvement (Jänig 1990)
-antidromic axonic flow of excitatory substances (Yamamoto et al. 1993)
-remodelling of the axolemma ionic channels (Devor 1994).

B) Central nervous system mechanisms. Reported medullary mechanisms of neuropathic pain are:

-spontaneous discharges caused by hyperexcitation of dorsal horn neurons following deafferentiation of dorsal roots or spinal nerves (Loeser et al. 1967, Basbaum et al. 1976)
-unbalanced control of spinal integration following excitotoxic damage of the inhibitory circuits (Wall et al. 1981, Sugimoto et al. 1990)
-development of new synapses, (Snow et al. 1989, Woolf et al. 1992)
-and/or activation of silent synapses (Devor et al. 1981)
-alterations of peripheral neurotransmitters or neuropeptides (Bennett et al. 1989).

C) Central sensitization and neuroplasticity.

-various CNS changes, induced by peripheral nerve lesions, can generate and/or amplify pain which persists after healing of the primary lesion. These changes are mainly induced in nervous system and wide dynamic range neurons of pain pathways by prolonged discharges of C-fibres (Woolf 1983)
-neural degeneration (Sugimoto et al. 1990)
-lowering of the threshold, frequency-dependent increase in amplitude and rate of discharges (wind-up phenomenon), prolonged after-discharges, wider peripheral receptive areas (McMahon et al. 1984)
-recruitment of new excitatory synapses (Cook et al. 1987)
-prolonged survival of excitatory synapses after cessation of afferent hyperstimulation (Woolf et al. 1986)
-such central changes may also be maintained by afferent pathways not belonging to the damaged area (Gracely et al. 1992) showing the feature of neuroplastic change (Pockett 1995).

D) Excitation of nociceptive neurons appears to be possible by the following neuro-active substances.

- C-fibre neuropeptides, like substance P (SP) (Chang et al. 1970, Randic et al. 1977, Moochala et al. 1984), neurokinins A and B, calcitonin gene-related peptide (Saria et al. 1986), vasoactive intestinal peptide, colecistokinine, somatostatin, galanin (Villar et al. 1991).
- Excitatory amino acids (EAA), mainly L-aspartate and L-glutamate (Curtis et al. 1960, Coderre et al. 1991, Wilcox 1991) which activate ionotropic, metabotropic, N-methyl-D-aspartic acid (NMDA) and non-NMDA receptors (Gerber et al. 1989). Activation of NMDA receptors connected with Ca^{2+} channels is an important mechanism of neuropathic pain (Woolf et al. 1991). Their inhibition reduces neuropathic pain (Aanonsen et al. 1986,Gordh et al. 1992).
- Nitric oxide, NO, is generated after activation of NMDA receptors by the neuronal nitric oxide synthetase of the dorsal horns of spinal cord. NO can potentiate other receptor-mediated central sensitization, activating many postsynaptic and also probably presynaptic biochemical processes (Garthwaite et al. 1988, Sanders et al. 1992, Meller et al. 1993, Lowenstein et al. 1994). It was shown in experimental models that intrathecal injection of NO donors induces hyperalgesia (Kitto et al. 1992), while inhibitors of NO synthesis (L-NAME, L-NMMA) prevent hyperalgesia induced by subcutaneous formalin (Malmberg et al. 1993), or by intrathecal glutamate agonists (Meller et al. 1996), or SP (Radakrishnan et al. 1995).
- EAA or SP bound to their receptors activate neuronal cyclooxygenase which synthesizes many substances, mainly medullary prostaglandins (PGE_2, PGD_2, PGF_2), which amplify intra- and extra-cellular pain transmission. Intrathecal injection of different substances produced by cyclooxygenase induces hyperalgesia in experimental models (Uda et al. 1990) while cyclooxygenase inhibitors reverse hyperalgesia induced by subcutaneous formalin (Malmberg et al. 1995) or by intrathecal glutamate and SP (Malmberg et al. 1992).
- EAA plus SP cause sensitization of the dorsal horn neurons by repetitive stimulation of afferent C-fibres.

This phenomenon appears due to summation of two post-synaptic depolarizing currents: the first follows activation of NMDA receptors. The second appears maintained by SP acting on NK-1 receptors (Gerber et al. 1989). Neuropeptides and EAA are co-localized in nociceptive afferent endings (Coderre et al. 1993). Their simultaneous release allows a reciprocal potentiation. Experimental data (Mjellem et al. 1992, Randic et al. 1990) demonstrated that only the simultaneous activation of NMDA and SP can induce neuronal sensitization, if the stimulus has adequate intensity and duration.

The final common result of NMDA and NK-1 receptor activation is the increase of intracellular ionised free calcium (Priebe et al. 1997), which could explain persistent neuronal hyperexcitability. Such receptorial activation is also able to activate protein-kinase C (PKC) via phospho-inositols cascade; many studies confirm the role of PKC in increasing and prolonging neuronal hyperexcitability (Coderre 1992, Chen et al. 1992).

An important outcome of central sensitization is neuronal plasticity: short- and even long-term changes in gene expression induced by extraneuronal stimuli (Woolf 1983, Hunt et al. 1987). For instance, thermal or chemical skin lesions induce an increase

of cytoplasmic concentrations of c-Fos and c-Myc (Hughes et al. 1995). A series of neuronal intracellular biochemical events modify the genetic expression, and this may change the characteristics of these cells for a very long time (Zimmermann 1993, Hughes et al. 1995). EAA play an important role in inducing c-Fos expression (Lerea et al. 1992) since intrathecal MK-801 decrease c-Fos in the medullary dorsal neurons (Kehl et al. 1991). Morphine decreases medullary expression of c-Fos in a dose-dependent way (Presley et al. 1990). SP plays a minor role in the dynamics of genetic derepression (Naranjo et al. 1991).

Experimental data suggest an important role of glial cells in the modulation of these neuronal events (Watkins et al. 1997). Glial cells, stimulated by EAA and SP, can release NO, EAA, cyclooxygenase products and some cytokines (IL-1, TNF-α, C3, NGF) in the extracellular space, close to the neuronal surface.

Many studies over the last years have produced evidence that sensitization of sympathetic nerve endings causes vasoconstriction, and that sensitization of other neuronal mechanisms leads to neuropathic pain. These two components, vasoconstriction and neuropathic pain, appear to be the two essential components of CRPS.

Chapter 4 THERAPEUTIC MODES IN TREATMENT OF CRPS

Few syndromes have so many reports on so many different types of treatment. Allen et al. (1999) published data about the clinical history of 51 CRPS patients, each receiving an average of five different types of treatment. The most common of these were; physical therapy (88%), nerve blocks (82%), tricyclic antidepressant medication (78%), opiate medication (70%), anticonvulsant medication (60%) and psychological treatments (50%). Many of the proposed methods for treating CRPS are directed toward interrupting the existing pathological cycle, for which blocking the sympathetic innervation was for a long time the most common treatment of CRPS (Ochoa et al., 1996).

4.1 Blocking sympathetic innervation

According to Zimmerman (1979), sympathetic reflexes sometimes function as a positive feedback and may increase the effect of a noxious stimulus. The relationship between sympathetic systems and painful conditions is well described by Gross (1974), Loh et al. (1978) and Nathan (1980). Sympathetic reflexes acting by a positive feedback may cause CRPS (Bonica 1953, Procacci et al. 1976, Bentley et al. 1980) and sympathetic blocks constitute a primary and effective treatment (Bonica 1953, Loh et al. 1978).

These cycles can be interrupted surgically (Evans 1926, Brücke 1946), or percutaneously (Pernak 1988), or by pharmacological blockade by means of a regional intravenous sympathetic block (RIS block) (Table VI). For decades, interruption of the impulses along the sympathetic nerves has been described as a therapy in CRPS complaints, based on the Lovén reflex. In 1866 Christian Lovén described a reflex (local vasodilatation with generalized vasoconstriction) which since then bears his name. In stimulating the posterior auricular nerve of the rabbit he observed vasodilatation in the ear on the same side. This could be prevented by cutting the cervical sympathetic chain. He also found that when a peripheral nerve in the hind leg was stimulated, the blood volume of the limb increased. Bayliss (1923) studied the Lovén reflex and found that the dilatation was due both to the excitation of dilatators in the dorsal roots and the inhibition of constrictors in the sympathetic outflow.

In 1930 Spurling published the first report on treating a patient with causalgia by surgical interruption of the cervicothoracic sympathetic chain.

Table VI. Blocking sympathetic innervation.

Surgical	Stellate ganglionectomy
	Lumbar sympathetic chain interruption
Percutaneously	Radiofrequency
	Phenol injection
	Alcohol injection
Regional intravenous sympathetic block	Guanethidine
	Bretylium tosylate

Surgical ganglionectomy, (Heyman 1924, Pearl 1937, Schumacher 1947, Lofstrum 1979), both cervical and lumbar, preceded newer percutaneous techniques which have the same blocking properties. When using neurolytic drugs, phenol (Reid et al. 1970) is reported to have advantages over alcohol (Boas et al. 1983). The neurolytic effect of phenol on nerve fibre conductivity was studied by Gregg et al. (1985). They found a greater neurolytic action by increasing the concentration of phenol from 6–9–12%. Spontaneous regeneration occurred rapidly and functional repair was almost complete in all fibres after eight weeks. Probably one of the best sympathetic blocking procedures is by means of percutaneous radiofrequency lesion, as described by Pernak et al. (1985).

A regional sympathetic blockade can be achieved surgically by paravertebral sympathetic ganglion blockade (Betcher et al. 1953) but also with intravenous guanethidine. The technique was originally described by Hannington-Kiff in 1974, and is essentially a modification of the regional intravenous procedure for inducing local analgesia. Pharmacologically, guanethidine functions as a false transmitter, being actively taken up by sympathetic nerve endings and then releasing norepinephrine from its storage sites. Thomson et al. (1982) have documented a threefold rise in total blood flow in the forearm following a sympathetic block with intravenous regional guanethidine, and the changes persisted for 48–72 hours. Since no associated increase in muscular blood flow was reported, they concluded that the rise was secondary to augmented skin blood flow, as confirmed by concomitant elevation of the skin temperature. Successful treatment of CRPS with intravenous regional reserpine has been reported by Benzon et al. (1980). Its application can also be via the Bier's method. Reserpine decreases the re-uptake of catecholamines by storage vesicles and depletes slowly norepinephrine stores in sympathetic nerve endings.

Based on the theory that CRPS treatment can respond to prolonged sympatholysis using an intravenous regional technique, Ford et al. (1988) described the effect of bretylium tosylate. This quaternary ammonium compound is an adrenergic blocking agent with actions similar to guanethidine. Bonelli et al. (1983) reported that guanethidine blockade of the peripheral nerve endings was more effective than blocking the stellate ganglion with bupivacaine. They stated that the effect of the intravenous guanethidine blockade lasts longer and is superior to the effect of a stellate ganglion blockade. Bonelli and colleagues (1983) showed that the CRPS group treated with guanethidine had symptoms for 6.6 $\pm$ 3.9 (SD) months (n=10) and the group treated with sympathetic nerve blockade had symptoms for 17.7 $\pm$ 14.9 (SD) months (n=9). But it is important to realise that the two groups showed different symptoms/signs probably due to the fact that the stage of the disease was different. Others (Farcot et al. 1981, Jacqnemoud et al. 1981) also reported good results with guanethidine blockade in CRPS patients, but remarked that patients with peripheral nerve lesions or those treated in a late stage had poorer results than patients treated earlier and without peripheral nerve lesions. McKain et al. (1983) evaluated the sympatholytic effects of guanethidine and reserpine in asymptomatic volunteers. They concluded that guanethidine produced selective adrenergic blockade with resultant peripheral vasodilatation. So it appears that guanethidine may be useful in the treatment of pure vasospastic conditions. Despite these promising results, Glyn et al. (1981) showed earlier that this guanethidine block did not block the sudomotor (cholinergic) sympathetic activity.

Casale et al. (1992) proved that analgesia following "Biers's" block is most likely

due to the ischemic blockade of sensory A fibres. Nowadays it appears unwise to create half an hour of ischemia on a manifest CRPS extremity. Ischemia, and its metabolic consequences, may play an important role in the pathogenesis of CRPS. As will later be explained, damage by the free radicals promotes the continuation of CRPS.

4.2 Physical therapy

While some forms of physical and occupational therapy are essential in the treatment of CRPS, used injudiciously they can aggravate the symptoms. While several forms of therapy involve heat or ice packs, extremes of temperature should be avoided, as this may increase afferent transmission and exacerbate the condition. The goal of therapy should be an active range of motion of the involved joint. It is hazardous to intensify the pain, swelling or stiffness by forcing motion to the point of discomfort. Especially in patients with CRPS in the area of the hand, splinting therapy is essential to prevent the development of contracture. It is important that the affected extremity is rehabilitated and not condemned to inactivity. Frazer (1978) reported that deep friction massage could be of value in the treatment. When the pain worsens, repeated local anesthetic blocks can help in facilitating the use of physical therapy, especially in case of very painful CRPS. The nerves in the plexus can be blocked by the epidural approach.

4.3 Electrical nerve stimulation

Electrical stimulation techniques can be employed in three different ways: transcutaneous, by stimulation of the dorsal spinal cord or of the peripheral nerve. Accumulated clinical and experimental data indicate that the sensation of pain involves central pathways which are also responsive to non-nociceptive stimuli. Stimulation of large myelinated sensory fibres with a small myeline sheath exerts a segmental inhibitory effect on input to certain dorsal horn interneurons in the cat (Wall and Cronly-Dillon 1960, Wall and Devor 1983). These interneurons are also activated by impulses in small diameter afferent fibres responsive to noxious stimuli. Selective stimulation of peripheral nerve causes pain only when the threshold for small diameter afferents has been exceeded, while at lower stimulus intensities only non-painful paraesthesia is obtained (Collins et al. 1960). Thus, the possibility of suppressing pain by selective large fibre stimulation arose. Consistent with Melzack and Wall's "Gate Control" theory (1965), clinical studies (Wall and Sweet 1977) demonstrated dramatic relief of pain using this technique. Stimulation of the dorsal columns, which presumably is selective for the central projections of larger diameter afferents, has also been reported to relieve pain (Shealy et al. 1970).

4.3.1 Transcutaneous electrical nerve stimulation

Several authors, including Steinbrocker et al. (1958), Goodman (1971), Stilz et al. (1977) and Richlin et al. (1978) reported good results using transcutaneous electrical nerve stimulation (TENS) in patients with CRPS in the area of the shoulder and hand. Although its exact mechanism of action is unknown, Melzack in 1971 and in 1975

speculated that TENS at levels capable of producing cutaneous tingling, could activate transmission in both large and small nerve fibres; interfering with the pain mechanism. However, others reported worsening of the CRPS by use of the transcutaneous stimulation method. Abram (1976) pointed out that the sympathetic activity could be increased by TENS. Wall (1964), Meyer et al. (1972) and Bohm (1978) reported good results in CRPS patients with transcutaneous electrical nerve stimulation treatment. An explanation for the effectiveness of TENS is perhaps that this technique activates an afferent population which has inhibitory actions on spinal nociceptive neurons of which the electrical stimulation causes suppression of ongoing, abnormal afferent activity. This explanation is suggested by the experimental results showing stimulus-induced suppression of spontaneous activity originating in the dorsal root ganglion of rats with neuromas (Burchiel 1984). The inconsistent results of Loeser et al. 1975, Ray 1975 and Eriksson 1979, showing success percentages ranging from 12.5% to 60% justify continued study of TENS in CRPS patients.

4.3.2 Dorsal spinal cord stimulation

This method of pain control was first introduced by Shealy in 1970, and is based on the "Gate Control" theory of Melzak and Wall (1965). The spinal cord stimulation electrodes can be used percutaneously or as a total implant. The best indications are reported to be persistent low back pain after failed surgery, and severe ischemic diseases of the lower extremities.

4.3.3 Peripheral nerve stimulation

An interesting study was presented by Racz and colleagues (1989), describing their experience with peripheral nerve stimulator implants for treatment of CRPS patients. They implanted electrodes under the affected nerve proximal to the site of injury, in cases of peripheral nerve damage causing causalgia. They treated 23 patients with this direct stimulation technique. Time from injury to implant ranged from 5 months to 10 years. They achieved good results rated on a linear pain scale; 2 of 11 females and 6 of 12 males returned to work.

4.4 Steroids

Poplawski et al. (1983) reported their results with treatment by way of intravenous regional blockades with lidocaine plus 80 mg methylprednisolone. They reported good results in the early stage, within six months after the initiating trauma. Glick and Helal (1976) also concluded that therapy with steroids is more successful when it is started at the onset of CRPS, and the treatment must be continued for several months. Systemic corticosteroids have been used since 1953 in the treatment of CRPS (Steinbrocker and Argyros 1958). The mechanism of action of corticosteroids in the treatment of CRPS is unknown, although several possible explanations have been offered. Anti-inflammatory activity against perivascular inflammatory infiltrate is, according to Kozin et al. (1976 a,b), a possible explanation. Another hypothesis is that corticosteroids act by their membrane stabilising effect. By this way they reduce capillary permeability and therefore decrease the plasma extravasation that is commonly associated with the early

state of CRPS. Christensen and coworkers (1982) performed a placebo controlled study on CRPS patients using systemic corticosteroids and placebo. The results showed 75% improvement with corticosteroids; the intergroup difference between corticosteroids and placebo was statistically significant ($p < 0.01$).

4.5 Calcitonin

From the wide range of available therapeutic approaches, Gobelet (1984) investigated calcitonin in CRPS patients. Calcitonin showed anti-osteolytic, analgetic and vascular regulatory properties. Ginsberg (1984) reported good results on pain components in 80% of the patients. Krause (1984) reported 72% successful results in stages one and two of CRPS but only 20% success in stage three of CRPS. Doury et al. (1981) and Toussanis (1984) reported good results only in stage one of CRPS.

4.6 Dimethyl sulfoxide (DMSO)

According to the theory that oxygen radicals play an important role in the pathogenesis of CRPS, some Dutch investigators claim a good effect with DMSO application of the affected limb (Goris et al. 1987, Zuurmond et al. 1996). The CRPS patients in Goris' study underwent treatment with DMSO 50% in water applied to the involved extremity five times daily for 10 minutes. In 7 of the 23 CRPS patients they found the cause of the pain triggering symptoms; these 7 patients had to undergo corrective surgery. They evaluated the change in condition of the affected extremity, in a placebo controlled study, by the range of motion. DMSO was the most effective treatment as to improvement of ROM ($p=0.035$) and as to overall improvement ($p=0.001$). The CRPS patients in the group of Zuurmond ($n=38$, mean age 50 ± 19 SD years) had no time delay longer than 3 months between the initial trauma and start of the treatment. Pain score was done by a visual analog scale (VAS). Results after a mean treatment period of 3.4 ± 1.9 (SD) months, showed a statistically significant positive result of an improved VAS score from 5.3 ± 2.9 (SD) to 0.9 ± 1.3 ($n=38$).

4.7 Other medicaments

In therapeutic histories of CRPS patients one can find use of analgesics for pain treatment: beta-adrenergic blocking drugs, alpha-adrenergic blocking drugs, calcium channel blocking drugs for circulatory corrections, vitamins as neurotropic drugs or oxygen scavengers, tranquillizers, antidepressants, and phenytoin (Swerdlow 1984, Smith et al. 1988, Chaturvedi 1989). The use of phenytoin has a reasonable theoretical background concerning the possible etiology of CRPS. Phenytoin is known to regulate and/or stabilise abnormal hyperexcitability in both peripheral and central neurons (Swerdlow 1984, Smith et al. 1988) and has been shown to facilitate inhibitory mechanisms (Fromm et al. 1982). Pharmacological agents are given in the list in Table VII.

Table VII. Drugs used in CRPS treatments.

Antidepressants	Paoli et al. 1960, Woodforde et al. 1965, Watson et al. 1992, Max 1994
Anticonvulsants	Rosner et al. 1966, Killian et al. 1968, Yaari et al. 1985, Burchiel 1988, McQuay et al. 1995
Clonidine	Coventry et al. 1989, Davis et al. 1991, Puke et al. 1993, Yamamoto et al. 1996
Echinacin	Birkenfeld 1954
Lidocaine i.v.	Boas et al. 1982, Rowbotham et al. 1991, Marchettini et al. 1992
Mexiletine	Daggered et al. 1988, Chabal et al. 1992
NSAIDs	Moncada et al. 1979, Yaksh 1982, Max et al. 1988, Malmberg et al. 1992, Malmberg et al. 1993, Eisenach 1993, Malmberg et al. 1995
Nifedipine	Prough et al. 1985
Opioids	Dickenson et al. 1987, Arner et al. 1988, Portenoy et al. 1990, Arner et al. 1993, Kolesnikov et al. 1993 , Mao et al. 1994, Mayer et al. 1995
Padutin	Roland 1952
Phenoxybenzamine	Muizelaar et al. 1997
Phenothiazine	Sigwald et al. 1957, Taub 1973, Davis 1977, Logan 1983
Phentolamine	Arner 1991, Raja et al. 1991

4.8 Conclusions

Unfortunately, despite considerable investigation into the pathology, etiology and treatment of CRPS the established condition remains a serious problem, is often disabling and presents a major challenge in treatment. The pathogenesis of CRPS in any given patient may be related to both peripheral and centrally-mediated factors, and this implies that a variety of treatment modalities may, to some extent, be effective. In addition, CRPS can develop through several stages over a period of time. One particular type of treatment will not necessarily be successful in every stage of the CRPS. One of the most important factors in predicting improvement with treatments is a short interval (less than six months) between the onset of CRPS symptoms and the administration of therapy. In many cases CRPS is not diagnosed in its early stages, for instance by misdiagnosis. It is generally believed that early recognition and treatment of the peculiar reflex originating from the site of trauma may successfully abort the late sequelae and the long-lasting disability by instituting prophylactic and simple therapeutic measures.

As Novelli et al. in 1998 stated, clinical trials combining different drugs, but following a rational basis, are needed to improve the therapies in CRPS.

Chapter 5 OBJECTIVE MEASUREMENTS OF CRPS SYMPTOMS: PLETHYSMOGRAPHY, SKIN TEMPERATURE AND PULSE OXIMETRY

5.1 Photo-electric plethysmography

In CRPS the clinical state is nearly always described as a complex of symptoms (in this work: pain at rest, pain on movement, impaired mobility, edema, hyperhidrosis, abnormal skin temperature and hyperpathia and/or allodynia). These symptoms are difficult to handle in an objective way. There is lack of consensus and no objective scales exist for the degree of severity of the symptoms. A way to assess the severity of the symptoms in each patient is to monitor the changes in their complaints and conditions over time.

From the viewpoint that CRPS is a circulatory disorder and linked to changes in the circulating volume of the affected extremity and associated with temperature changes of the skin, monitoring the pulsations associated with changes in blood volume in a peripheral vascular bed with photo-electric plethysmography, is an effective tool (Dorlas et al. 1985).

The basic principle behind the method, first reported by Herzman (1938), is relatively simple. A small light source and a photosensitive detector (a photo-electric cell) are attached to an appropriate part of the skin. The emitted light is scattered and partly absorbed in the tissues. Another part of the light emerges through the skin and is detected by the photo-electric cell. The intensity of the light is determined by the optical density of the solid tissues such as skin, connective tissue and bone, and by the varying amount of blood in the vascular bed (Weinman and Manoach, 1962). Blood has higher light absorption coefficient than the surrounding tissues (Kramer et al. 1951, Zijlstra and Mook 1962), so that increases in the amount of blood lead to a decrease in the total amount of light detected (Challoner and Ramsey, 1974; Challoner, 1979).

For interpretation of the photo-electric plethysmogram, it should be realised that those changes in light are measured which are proportional to the total amount of light detected and which cannot be calibrated (Nijboer et al. 1981). Therefore, the amplitude of the photo-electric plethysmogram does not indicate the height of the arterial pulse waves in a quantitative way. Nevertheless, several authors (Elings, 1959; Herzman, 1959; Zijlstra and Mook, 1962) have reported a good correlation between finger photo-plethysmographic amplitude and the blood flow in the finger. Dorlas and Nijboer (1985) compared photo-electric plethysmography with mercury-in-rubber strain-gauge plethysmography, the latter without applying venous occlusion, so that only the arterial blood volume pulsations were measured. The changes in amplitude of both plethysmograms proved to be identical in 98% of the total number of changes in 104 patients (Nijboer et al. 1983). This means that changes in the amplitude of the photo-electric plethysmogram, just like the blood volume pulsations (ΔV), depend on the distensibility of the vascular wall (D) as well as on the intravascular pulse pressure (ΔP). Their relationship was given by Burton (1972) as $\Delta V = D.\Delta P$. In the peripheral arterial bed, the

distensibility factor depends mainly upon the tone of vascular smooth muscle, which is controlled by the autonomic nervous system, which in its turn is dependent on metabolic factors. As a result of the special mechanical arrangement of vascular smooth muscle fibres (Burton, 1954), the effect of autonomic impulses upon distensibility will be so strong that it completely predominates over the effect of pulse pressure in several regions of the body.

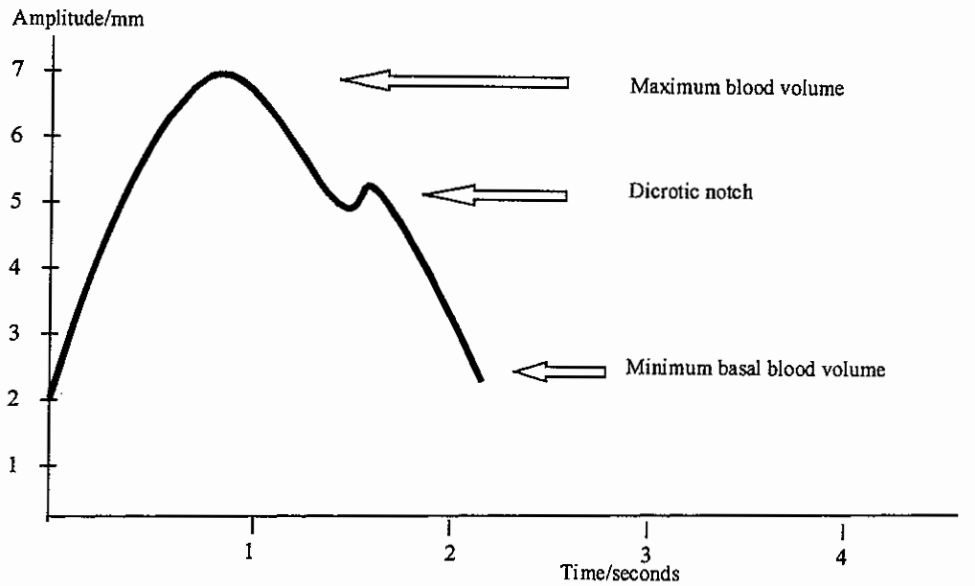

Fig. 1. The normal pulse photo electric-plethysmographic wave form.

Figure 1 shows the pulse-photo-electric plethysmogram wave form. The excursions are synchronous with the heart beat, which arise because volume distensions of the root of the aorta are propagated along the walls of the vascular tree to the terminal arterial bed from which the plethysmogram is recorded. The maximum amplitude corresponds with the maximum blood volume and not with blood pressure. Amplitude is the result of cardiac output, volume loading and vasomotor tone. The area under the curve is indicative of left ventricular stroke volume. Respiration alters the peripheral pulse wave and the baseline. In the descending run-off phase of arterial pressure and peripheral pulse wave is a dicrotic notch or incisura, classically attributed to closure of the aortic valve at the end of ventricular systole. Whether this interpretation is always applicable in the peripheral pulse wave form is questioned. The vertical position of the incisura on the pulse wave might be used as an indicator of vasomotor tone: under most circumstances the notch descends to the baseline during increasing vasodilatation and climbs towards the apex with vasoconstriction. Full interpretation of the data available from the peripheral pulse wave as plethysmographic recording may give rise to important additional diagnostic information (Murray et al. 1996).

The arterial pulse wave is generally used for monitoring purposes (Elings 1959) because its amplitude indicates the arterial blood flow pulsations reaching the periphery. Especially when taking a trend recording speed for the plethysmographic recording,

changes in peripheral blood flow can be recorded in a way that allows to visualise the changes in blood flow induced by changes in the regulatory mechanism of the autonomic nervous system.

5.2 Skin temperature measurements

Simultaneously with the plethysmogram, the skin temperature can be recorded. There was a widespread belief that vasoconstriction, seen on the plethysmogram, was the result of cooling of the skin when exposed to the comparatively cool surrounding temperature in the operating theatre (Otteni, et al. 1970). However, Dorlas and Nijboer (1985) studying 40 patients in whom skin temperature was measured during anesthesia (Fig. 2), showed that the decrease in amplitude of the plethysmogram always preceded the decrease in finger temperature (Dorlas 1974; Nijboer et al. 1983).

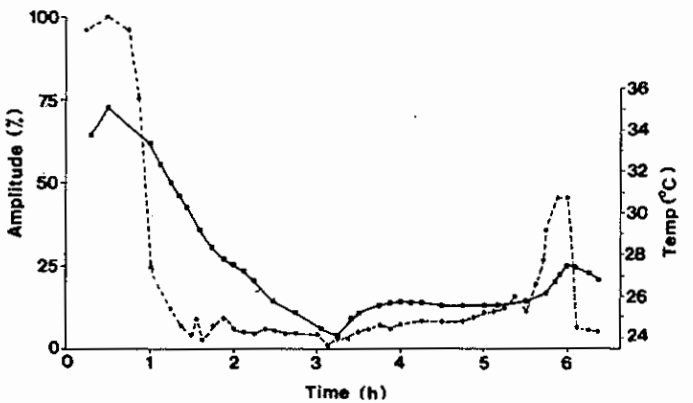

Fig. 2. Photo-electric plethysmography as a monitoring device in anaesthesia. Reproduced from, Dorlas JC, Nijboer JA. Br J Anaesth 1985; 57: 542–530 (with permission). Illustration of the changes in plethysmographic amplitude (•- - -•) preceding the changes in skin temperature (——) when recorded simultaneously from a finger during surgery.

Therefore, the decrease in finger temperature is the result of, and not the cause of, the vasoconstriction. Because of the link between the two phenomena, we recorded both plethysmogram changes and temperature changes, whilst studying the effects of pharmacological agents on patients.

5.3 Pulse oximetry

The ability to use pulsatile light variation to measure arterial oxygenation was first recognised by the Japanese physiological bioengineer Takuo Aoyagi in the early 1970s (Rheineck Leyssius, 1998). Pulse oximeters use two different frequencies and as a consequence only two substances can be determined. Pulse oximeters might use wavelengths between 600 nm and 1300 nm, outside this window the energy absorption by the

tissue is too high. The differential absorbance by HbO_2 and Hb should be high and the chosen wavelengths should lie on the left and right side of an isobestic point (isobestic = equal absorbance for Hb and HbO_2 [805 nm]). So, pulse oximeters, for practical reasons, use only two wavelengths. Only under ideal conditions (as during calibrations in healthy non-smokers) does pulse oximetry measure functional arterial oxygen saturation (SaO_2). Pulse oximeters calculate the ratio, S of the pulsatile absorbance (AC) to the non-pulsatile absorbance (DC) at the wavelengths of 660 nm and 940 nm:

$S(660) = AC(660)/DC(660)$

$S(940) = AC(940)/DC(940)$

The instrument then calculates the ratio of these two 'pulse-added absorbance' signals:

$R = S(660)/S(940)$

This value of R is used to find the pulse oximeter oxygen saturation (SpO_2) in a table built into the oximeter software. The ratios can be approximately determined on a theoretical basis, but for accurate predictions of SaO_2 experimental data in human volunteers are required. The ratio R varies from approximately 0.4 at 100% haemoglobin saturation to 3.4 at 0% haemoglobin saturation.

Haemoglobin provides the oxygen transport capacity of blood and maintains the driving force for diffusion of oxygen through the interstitial fluid to the cells in a small range. The oxygen transport capacity is generally described by the following equations:

O_2 transport capacity (ml $O_2.min^{-2}$) = cardiac output x arterial O_2 content

O_2 content [ml O_2 . (100 ml blood)$^{-1}$] = $2.14 \times Hb \times SaO_2 + 0.003 \times PaO_2$

Hb is expressed in mmol.l^{-1} and the oxygen pressure of arterial blood (PaO_2) in mm Hg. The oxygen pressure of arterial blood is 90–100 mm Hg and haemoglobin is 98–99% saturated with oxygen. The arterial O_2 content is about 20 ml.(100 ml)$^{-1}$ blood; 1.5% of O_2 is dissolved in plasma and 98.5% is bound to Hb. Normally, the oxygen pressure of mixed venous blood is 40 mm Hg and the haemoglobin is 70–75% saturated. The oxygen extraction ratio, which is (the arterial O_2 content minus the venous O_2 content) divided by the arterial O_2 content, is 25–30%. The venous oxygen pressure is equal to the oxygen pressure of the interstitial fluid near the capillaries and is the driving force for oxygen to diffuse from the capillaries to the mitochondria in the cells. Oxygen consumption may increase ten-fold during exercise, and extra oxygen will be delivered, in part, as a result of increased cardiac output and also by increased oxygen extraction from the haemoglobin, which results in a lower venous oxygen saturation. Thus not only a decrease in supply of oxygen leads to a decrease in SaO_2, but also an increase in oxygen consumption by the metabolism results in a decrease in SaO_2.

Chapter 6 HYPOTHESIS ON SEROTONIN EFFECTS ON PERIPHERAL CIRCULATION AND PAIN: MECHANISMS OF ACTION OF KETANSERIN

6.1 Serotonin and 5-HT receptors

Regulatory mechanisms at the level of the microcirculation are very complex and not completely known. The influences of drugs and mediators do not always have the same effects in vivo as they have in vitro and may be unpredictable because another confusing factor is that in these regulatory mechanisms, pathological influences do not always have the same disturbing properties. Superior higher regulatory mechanisms can compensate for the disturbed balance.

In the complex of peripheral microcirculation the endothelial cells play an important role both anatomically and physiologically. The endothelium acts as a barrier that excludes circulating cellular elements and harmful substances, and it produces modulators of the function of the blood cells, the vessel wall, and the surrounding tissue. The endothelial cells participate in capillary transport, plasma lipids and proteins concentration regulation, and control of hemostasis. Platelets interact both physically and biochemically with the blood vessel wall and the endothelium plays an important role in these interactions. Where the endothelium is damaged, platelets aggregate on the sub-endothelial collagen and smooth muscle. As an inevitable consequence of their aggregation, platelets release serotonin, nucleotides, prostaglandins, catecholamines and various proteins, all of which may have profound effects on other platelets, the surrounding endothelium, the smooth muscle, or the tissue beyond.

The effect of serotonin will depend on factors such as its concentration, the integrity of the endothelium and the degree of responsiveness of the vascular smooth muscle. The prejunctional inhibitory effect of serotonin on adrenergic neurotransmission is explained by the fact that the α-adrenergic receptors have the same affinity for serotonin as the 5-HT binding sites. In this way, serotonin has complex effects on the vascular system which can lead to vasoconstriction or vasodilatation (Van Nueten and Janssens 1986).

Serotonin causes vasoconstriction by 1) direct activation of 5-HT_{2A} receptors on the smooth muscle cells, 2) activation of serotonin release from platelets (van Nueten and Janssens 1986, Vanhoutte et al. 1984), 3) amplification of the vasoconstriction response by other neurohumoral mediators, such as norepinephrine or angiotensin II (Lecomte 1953), 4) amplification by activation of post-junctional adrenoceptors (Meehan et al. 1986), 5) displacement of stored norepinephrine from adrenergic nerve terminals which has an indirect sympathomimetic effect (Fig. 3).

The vasodilator action of serotonin is primarily mediated by the endothelial cells or can be due to inhibition of sympathetic neurotransmission (Van Nueten 1983): 1) by prejunctional inhibition of adrenergic neurotransmission, 2) activation of inhibitory autonomic nerves (Göthert et al. 1986), 3) 5-HT_{2C}/5-HT_{2B} receptors on endothelial cells trigger nitric oxide release (Fozard et al. 1994), 4) by activation of inhibitory serotonergic receptors (5-ht_7)on the smooth muscle cell (Hoyer et al. 1994) (Fig.4).

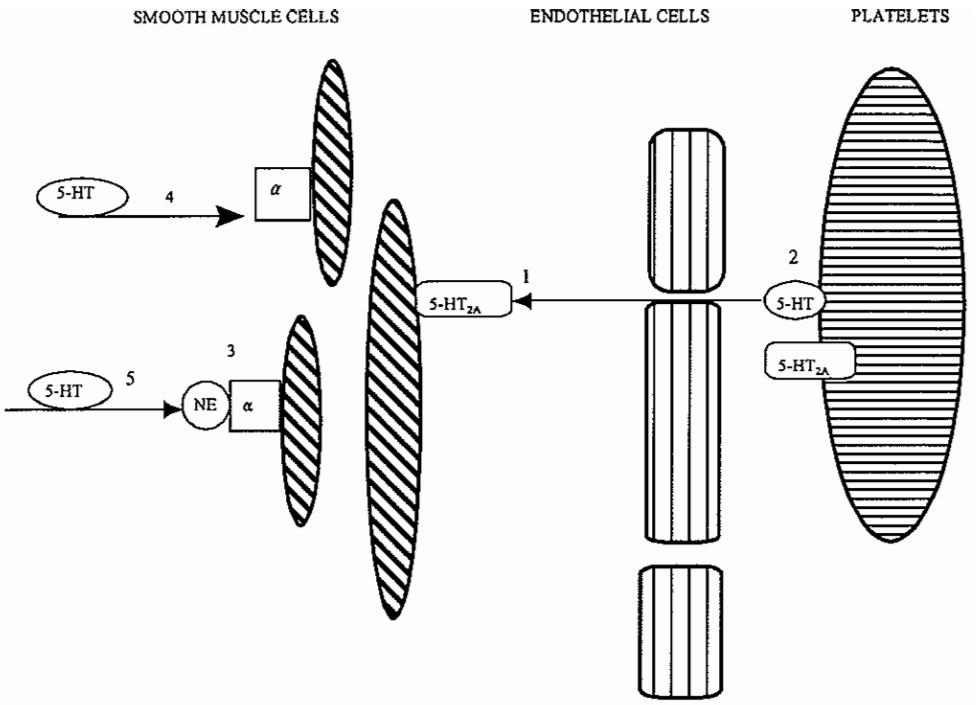

Fig. 3. Sites of action of serotonin in promoting vasoconstriction. The cells involved are drawn as striped structures, receptors as squares and hormones as small ovals or circles. See the text for further details.

The vasoconstrictor and vasodilatator effect of serotonin may balance out, but when the vasoconstrictor component predominates, vasospasm may occur resulting in a pathological condition. The individual sensitivity of various blood vessels to serotonin may vary considerably. Serotonin (e.g. released from aggregating platelets) also induces vascular contractions by amplifying the response to other vasoactive substances. The vascular reactivity to serotonin can be markedly augmented by acute hypoxia and by cooling. Blood vessels also can become hyperreactive to the vasoconstrictor component of serotonin in a number of disease states; for example, in cutaneous arteries in sclerodermic patients (Winkelman et al. 1976). Both the direct and indirect vasoconstrictor responses to serotonin, whether or not augmented by acute or chronic conditions, are inhibited by serotonin receptor antagonists (Van Nueten and Janssens 1986).

In 1949 Rapport identified the serum vasoconstricting agent serotonin as 5-hydroxytryptamine (5-HT). Synthetic serotonin showed to be highly active in many smooth muscle models (Erspamer 1954, Garatti and Valzelli 1965, Green et al. 1979). In both the human saphenous veins (Göthert et al. 1986) and in isolated rabbit ear arteries (Meehan et al. 1986) inhibitory presynaptic 5-HT receptors on the sympathetic nerves play an important role in the mechanism of vasoconstriction and vasodilatation. Two

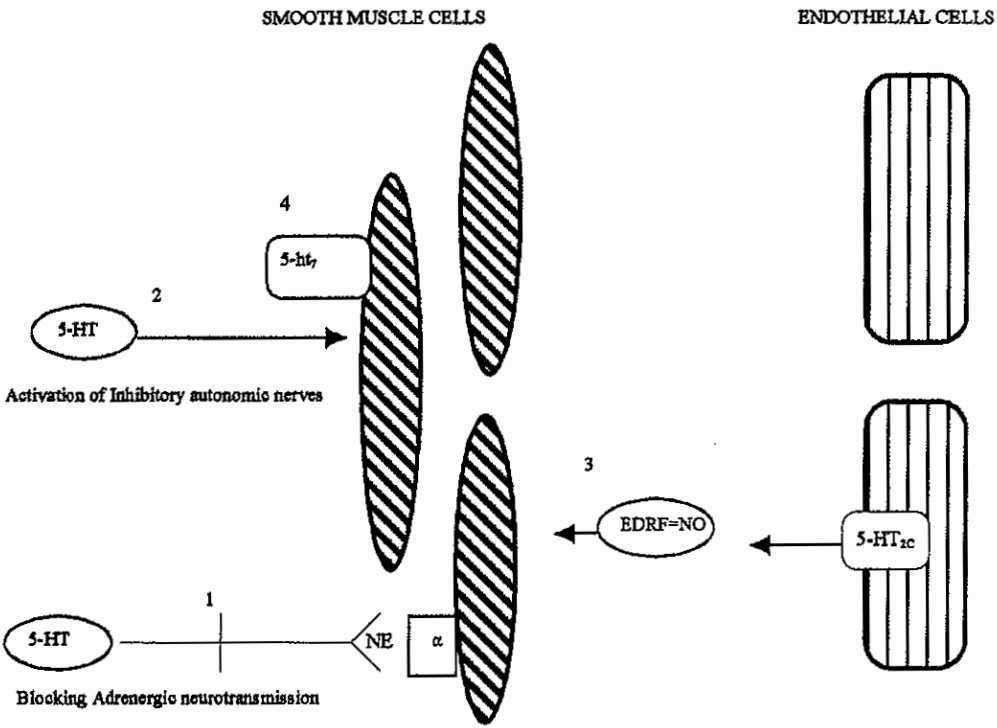

Fig. 4. Sites of action of serotonin in promoting vasodilatation. See legend of Fig.3.

distinct binding sites for serotonin have been identified in brain tissue and labelled 5-HT_1 and 5-HT_2 binding sites (Van Nueten et al. 1984,b).

Since the 5-HT receptors were being referred to by various names (D, M, 5-HT_1, 5-HT_2, S_1, S_2, and others), the need for a uniform terminology was advocated (Humphrey 1983, and Verdouw et al. 1984). In 1984 Saxena and Verdouw suggested to replace the 5-HT receptor classifications by a new classification based on a 5-HT_1, 5-HT_2-5-HT_n series. The 5-HT receptors subserving arteriolar dilatation, presynaptic inhibition of sympathetic transmission, autoinhibition in the brain and possibly constriction of arteriovenous anastomosis, were termed 5-HT_1. The receptors mediating vaso- and broncho-constriction and platelet aggregation were named 5-HT_2 receptors and those mediating ganglionic stimulation, the Bezold-Jarisch reflex and catecholamine release in the heart were designated as 5-HT_3 receptors (Saxena 1995).

Nowadays, serotonergic neurotransmission is recognized as a complex mechanism involving pre- and post-synaptic events and more distinct 5-HT receptor subtypes are classified. The "Nomenclature Committee of the Seretonine Club" has proposed the following classification and nomenclature: the main receptor types 5-HT_1 to 5-HT_4, recombinant receptors 5-ht_5 to 5-ht_7 and "orphan" receptors (Saxena 1995). Each group is not only operationally but also structurally distinct, with each receptor group having its own distinct transducing system (Hoyer et al. 1994). The 5-HT_{2A} receptor mediates mainly contraction of vascular and nonvascular smooth muscles, platelet aggregation and neuroexcitation (Saxena 1995). Ketanserin was characterised as a 5-HT_{2A} receptor antagonist.

6.2 Mechanism of action of ketanserin

Ketanserin is a quinazolinedione derivate with the following chemical structure (Fig. 5):

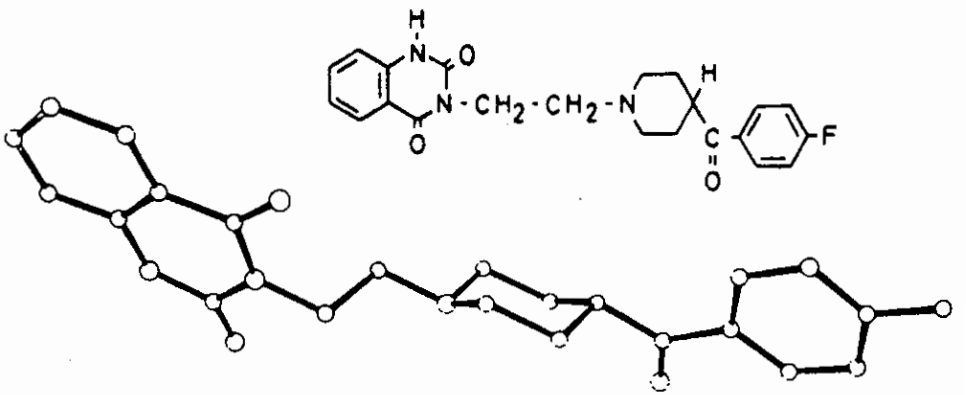

Fig. 5. Chemical and crystallographic structures of ketanserin. The tartrate salt of ketanserin, 3-{2-[4-(p-fluorobenzoyl) piperidino]ethyl}-2,4 -quinazolinedione, is a white powder that is soluble in water (Drawing and legends, Awouters 1985c).

Ketanserin is a very effective antagonist of endogenous serotonin. Ketanserin is a potent peripherally acting serotonin antagonist with weak alpha-adrenergic blocking and antihistamine activity (Awouters 1985a). Using different tissues and appropriate ligands, ketanserin was found to be inactive at alpha-two, and beta adrenergic, GABA muscarinic and opiate receptors, to have low affinity for D_2 dopamine receptors, and moderate affinity for alpha-adrenergic and histamine H_1-receptors (Leysen et al. 1981).

Ketanserin has been widely studied to establish its potency to antagonise serotonin-induced effects on laboratory preparations and in whole animals from different species (Awouters et al.1985b, Ball et al. 1983, Cantalamessa et al. 1983, Kalkman et al. 1982, Lindberg et al. 1984, Niemegeers et al. 1983, Reiche et al. 1983, Van Gerven et al. 1979, Van Nueten et al.1979,Van Reempts et al. 1981, van de Water et al. 1980, Yap et al. 1983,). The release of serotonin from platelets in rats induces prompt cyanosis of the ears and widespread tissue edema, and more slowly gastric lesions, all of which are inhibited by low doses of ketanserin (Forard et al. 1983, McLennan et al. 1983). In other common laboratory animals and in man, the vessel wall appears to be the primary target of circulating serotonin.

Saxena and Verdouw (1984) studied the effect of 5-hydroxytryptamine on the distribution of carotid blood flow into the capillary (nutrient) and arterio-venous anastomotic (AVA) fractions in anaesthetized pigs. They concluded that the 5-HT_2 receptors mediate vasoconstriction and are located in the large conducting arteries and possibly, in smaller numbers, in the AVAs and arterioles.

The pharmacological activity profile of ketanserin, when compared with various reference compounds points to potent, specific serotonin antagonism which is associated with weak histamine and adrenaline antagonism (Awouters 1985c). Part of vascular 5-HT_2 antagonism is, therefore, directly linked to processes such as platelet activation, the release of 5-HT from their dense granules, the vascular action of other platelet-

derived factors, the rate of 5-HT re-uptake by platelets and thrombi. Besides these directly linked influences of ketanserin on serotonin processes in relation to platelet activation, there may be other mechanisms related to other anatomical structures. In literature about the antihypertensive aspects of ketanserin the overall opinion reported by Saxena (1990) concludes to a blockade of 5-HT_{2A} receptor- or α_1 -adrenoceptor-mediated vasoconstriction, combined blockade of α_1 -adrenoceptors and 5-HT_{2A} receptor mediated amplification of noradrenaline vasoconstriction, inhibition of central vasomotor loci, and a "direct" vasodilatation. For the indication of peripheral circularory disorders, like in CRPS, we are not interested in antihypertensive properties of the drug, but only in initiating a better flow in the peripheral area of the affected extremity (see Fig 6). As shown in chapter 7 using ketanserin in a dose of 10 mg i.v. there is no clinically important decrease in blood pressure or increase in heart rate. Also in 8 healthy (non hypertensive female volunteers, chapter 10) a hypotension, as expression of α_1-receptor blockade was not seen. Van Nueten et al. (1986) suggested involvement of a combination of 5 HT2 receptor (responsible for the potentiation of noradrenaline-induced vasoconstriction) and α_1 -adrenoceptor blockade in the antihypertensive action of ketanserin. Kato et al. (1999) reported about the expression of subtypes of 5-hydroxytryptamine receptors in arteries and veins in rats. They found hat the arterial 5-HT_{2A} receptor mRNA level was 25-fold ($p<0.01$) higher than the venous level. This explains probably that in the revalidation period of CRPS, while using ketanserin, the better circulated extremity turns to cyanosis and edema again when the patient holds the extremity in a vertical position. The effect of ketanserin, as 5-HT_{2A} blokker, is probably in this phase stronger on the arterial side as on the venous side.

6.3 Ketanserin activities on the vessel wall

Larger isolated blood vessels generally respond with marked contraction to low concentrations of serotonin. Isolated platelets activated with thrombin or by making contact with the blood vessel wall cause a contraction of the blood vessel. Substantial contraction of the blood vessel wall has been shown in rat caudal artery (Van Nueten et al. 1984a), canine coronary artery (De Clerck and Van Nueten 1982), basilar and internal carotid artery (McGoon and Vanhoutte 1984), rabbit pulmonary artery (Moulds et al. 1984) and the human digital artery (Vanhoutte and Cohen 1984). Platelet-derived serotonin contributes to this contraction. Platelet-mediated vascular contractions are inhibited by ketanserin at concentrations that are needed to inhibit contractions induced on the same vascular tissues by exogenous serotonin (Van Nueten 1983, De Clerck et al. 1984, McGoon and Vanhoutte 1984), and therefore appear mediated by 5-HT_2 vascular receptors (Awouters 1985c). The complete absence of partial agonist activity with ketanserin may be the most basic requirement to respect normal vessel tone throughout the circulation and to obtain the largest possible inhibition of potential serotonergic overactivity.

Blauw et al. (1991) studied the effects of intra-arterially infused serotonin (5-HT) on capillary and arterio-venous anastomotic (AVA) blood flow in the hand and forearm of 19 healthy volunteers, using radioactive microspheres with a diameter of 15 μm. The 5-HT_2-receptor antagonist ketanserin was used in an attempt to identify the receptors involved. Ketanserin increased both total forearm blood flow and AVA blood flow. The

drug blunted the constrictor response to 5-HT in the forearm but only slightly attenuated this response in the finger. This inhibition refers to the contractile action of serotonin and not to serotonin mediated relaxation which is not affected by ketanserin. Serotonin not only markedly sensitizes vascular tissues to other circulating vasoconstrictors, but also produces enhanced vasoconstrictor responses in conditions of hypothermia, anoxia and endothelial damage. Based on these activities, the clinical potential of ketanserin may be promising in alleviating the symptoms in patients with CRPS.

6.4 Ketanserin activities in relation to platelet activation

Platelets are the most important source of serotonin in the circulation. Therefore the relevance of vascular 5-HT_2-antagonism is directly linked to processes such as platelet activation, the release of serotonin from their dense granules, the vascular action of other platelet-derived factors, the rate of serotonin re-uptake by platelets, and (micro) thrombosis (Awouters et al. 1985b).

Details of the platelet-related pharmacology of ketanserin have been described (De Clerck et al. 1982b, De Clerck et al. 1983, De Clerck et al. 1984, De Clerck and Reneman 1985). Serotonin activates 5-HT_2 receptors on blood platelets (De Clerck 1984b, Lamprignani et al. 1982). Platelet activation by serotonin may result in changes in shape and transient aggregation or higher degrees of activation, up to full aggregation. Maximal formation and release of mediators are observed with serotonin in particular species.

The platelets own serotonin may further propagate the activation and they increase the thrombus size by their secondary recruitment. Whatever the initial stimulus for aggregation may be, the released serotonin will affect vascular smooth muscle. Ketanserin is a potent inhibitor of the serotonin-induced activation. An important effect of ketanserin in CRPS patients may be its restoration of the normal circulation by the significant inhibition of platelet serotonin mediated vasospasm. Potent, direct antagonism of serotonin in vessels and platelets appears to require high affinity binding to 5-HT_2 receptors.

The vessel wall appears to be the primary target of circulating serotonin. Low doses of serotonin can sensitize vascular smooth muscle to the contractile action of adrenaline, histamine, prostaglandin F_2, angiotensin II, hypoxia and hypothermia. Ketanserin inhibits the amplified responses and is very effective against the mixture of vasoactive agents released from aggregating platelets (Awouters et al.1985b). This selective serotonin antagonism at the level of blood platelets and vessel walls should perhaps be regarded as a sufficient basis to eliminate the important vasoactive role of serotonin in the situation of CRPS patients.

6.5 Localisation in neuronal structures of 5-HT_{2A} receptors and the consequences of their role in the pain perception

From out the view that serotonin, besides circulatory flow effects, plays also an important role in peripheral pain mechanisms, Pierce et al. (1996), studied the differential distribution between the sensory and sympathetic nervous system of specific subtypes

of 5-HT receptors in rats. With the polymerase chain reaction they amplified and distinguished the DNAs coding for serotonin receptor subtypes: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_{5A}, 5-HT_{5B}, 5-HT_6 and 5-HT_7. In lumbar dorsal root ganglia, in the cervical superior ganglia as well in the lumbar sympathetic ganglia they found 5-HT_{2A} receptors.

Laporte et al. (1996) established the respective distribution of serotonin 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2A} and 5-HT_3 receptors at the cervical, thoracic and lumbar levels of the spinal cord from subjects who died aged 81–94 years. 5-HT_{2A} sites were labelled by [^{3}H] ketanserin and they were found in the dorsal horn of the cervical segments.

Tokunaga et al. (1998) investigated which subtype of 5-HT receptors were involved in 5-HT-induced hyperalgesia using behavioural assessment of hyperalgesia in the rat. They strongly suggested that the 5-HT_{2A} receptor subtype is involved in 5-HT-induced hyperalgesia in acute injury and inflammation in the rat. The anatomical base was founded by in situ hybridization histochemistry which revealed the presence of 5-HT_2 receptor mRNA in a subpopulation of both large and small neurons in the rat dorsal root ganglia.

Pierce et al. (1995) demonstrated that 5-HT-induced synovial plasma extravasation is mediated via 5-HT_{2A} receptor. They concluded that their findings suggest a role for 5-HT_{2A} antagonists in various synovial inflammatory pain states.

Baeken et al. (1998) developed a new radioligand with high affinity and selectivity for serotonin 5-HT_{2A} receptors. They studied the influence of age and gender on the ligand binding in healthy volunteers. No gender difference was demonstrated. 5-HT_{2A} binding was shown to decline with age. Over an age range of 40 years a reduction in ligand binding of 42%±7% was found. These results are in agreement with in vitro and positron emission tomography findings of a decline in 5-HT_{2A} receptor binding with age.

Abbott et al. (1996) investigated the pain response produced by alpha-methyl-5-HT plus prostaglandin E_2 in rats. They showed that the pain response was blocked by the 5-HT_{2A} antagonist ketanserin and they concluded on the basis of their data that a 5-HT_{2A} antagonist, ketanserin, may be effective as a peripherally acting analgesic or analgesic adjuncts in pain associated with 5-HT release from platelets, such as in acute injury and perhaps some other chronic pain states, in which case our thoughts of course turn to CRPS.

Chapter 7 TREATMENT OF CRPS PATIENTS WITH KETANSERIN

7.1 Effect of ketanserin on the photo-electric plethysmogram of CRPS patients

All patients fulfilled the criteria of at least four of the seven symptoms of CRPS: pain at rest, pain on movement, impaired mobility, edema, hyperhidrosis, abnormal skin temperature, hyperpathia and/or allodynia. In early studies on ketanserin treatment we used a Hewlett Packard monitoring device for the photo-electric plethysmogram trend recording. Later, in the carnitine studies a Datex monitor was used, in which the trend recording was specially modified for pain treatment evaluation. This latter device has the advantage of simultaneously recording the photo-electric plethysmogram and the skin temperature of the affected area, together with pulse oximetry.

We have published several reports on the effect of ketanserin on CRPS patients in the last decade. In these studies photo-electric plethysmography played an important role (Moesker et al. 1985, Moesker et al. 1986, Moesker 1991, Moesker 1995). Figure 6 shows that intravenous administration of 10 mg ketanserin in a CRPS patient causes a substantial widening of the plethysmogram. This reflects the effect of changing the distensibility of the peripheral arterial bed. Distensibility mainly depends on the tone of the vascular smooth muscle, which is controlled by the autonomic nervous system.

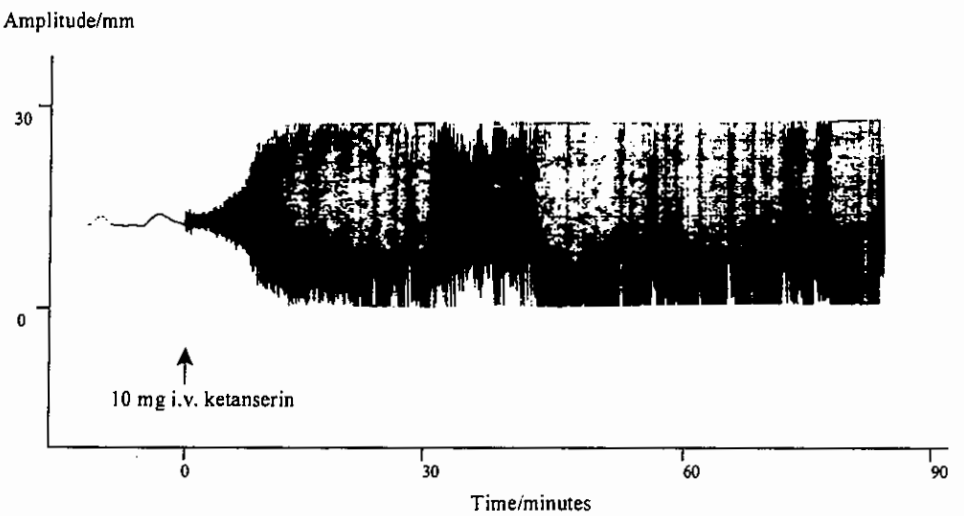

Fig. 6. Effect of 10 mg ketanserin on the photo-electric plethysmogram of the affected limb of a CRPS patient at a trend recording speed of 3.5 cm/hour (Hewlett Packard monitoring). The arrow represents the moment of ketanserin injection.

Because no significant changes in blood pressure were seen in our patients, the increase in amplitude of the plethysmogram must be the result of changes in the total amount of

detected light, which are predominantly determined by the volume changes of the venous bed. The symptom of edema therefore disappears very rapidly. Since the cutaneous veins are sympathetically innervated (Herzman 1959, Shepherd and Vanhoutte 1975) we can qualify the effect of ketanserin as vasodilatation by means of antagonising the serotonergic effect of the sympathetic neuro-transmitter norepinephrine.

7.2 Ketanserin treatment after failure of guanethidine treatment

CRPS patients in whom guanethidine regional intravenous blockade treatment failed were treated with ketanserin. In one year, 16 patients with CRPS were referred. In our pain clinic, as in similar clinics, these patients were treated with repeated guanethidine regional intravenous blocks. Six of the 16 patients recovered from the CRPS symptoms. This success rate of 36% is somewhat lower than generally reported, perhaps because in The Netherlands such patients are referred to a pain clinic at a later stage than elsewhere. The remaining 10 patients (mean age 40.1 years, mean duration of complaints 2.4 years) who failed to respond satisfactorily to guanethidine, were treated with 10 mg i.v. ketanserin followed by an oral maintenance treatment of ketanserin 60–80 mg daily, (given in three dosages of 20, 20, 20 mg or 20, 20, 40 mg). The rationale to treat these CRPS patients with ketanserin was partly based on theoretical grounds (Chapter 6), and partly on results of reported studies in other patients with diseases of peripheral vascular flow (Stranden et al. 1982). We assumed that sympathetic overactivity leads to an increased synthesis of various substances, such as bradykinin and serotonin, in the affected CRPS extremity. The effects of serotonin on the circulation received particular attention from our group. In the literature, ketanserin was reported to have promising results in the treatment of peripheral vascular diseases, such as intermittent claudication and Raynaud's phenomenon (Stranden et al. 1982). Based on those results, 5-HT_{2A} blockade could be an aid in treating CRPS patients; therefore, we decided to study its effects.

The data of the patients are summarized in Table VIII. All patients gave informed consent, and the intravenous treatment was performed after at least 15 minutes of acclimatisation in a temperature-stable (20–23°C) room. Ketanserin was administered i.v. as a slowly injected bolus into a running physiological saline infusion in a vein of the non-affected upper extremity.

Table VIII. Patient data: intravenous treatment followed by oral medication group.

Number of patients		10
Male/Female		6/4
Age (years) mean ± SD		40.1 ± 5.8
Duration of complaints (years) mean ± SD		2.4 ± 0.8
Sites of complaints - leg		6
	- arm	4
Etiology	- fracture	5
	- contusion	3
	- surgery wound	2

Immediately before and 30 minutes after each i.v. administration, the temperature of the affected limb was recorded with a Hewlett Packard electro-skin probe placed between

digits 4 and 5. Blood pressure and heart rate were recorded. The subjective symptoms were scored as normal, moderate, severe, or very severe. Statistical analysis of the differences in the values recorded before and after treatment within this group was performed using the Wilcoxon test with two-tailed probability. In all 10 patients, administration of 10 mg ketanserin i.v. prior to the oral maintenance therapy resulted in a significant increase in skin temperature from 27.9 to 30.8°C after 30 minutes ($p<0.01$).

In the following weeks the symptom of the skin temperature was evaluated separately from the other symptoms (Fig. 7). The rationale for this was that only this symptom can be measured exactly, without subjective influences, and because skin temperature correlates with changes in the cutaneous circulation. At baseline, in 6 patients the skin temperature was severely decreased and in 4 patients moderately decreased. After guanethidine application, 3 patients had a normal skin temperature, but 4 patients remained with a severely decreased and 3 with a moderately decreased skin temperature. After 6 weeks of oral ketanserin treatment 9 patients had a normalised skin temperature and only one patient still had a moderately decreased skin temperature, after 12 weeks the skin temperature of this latter patient also normalised.

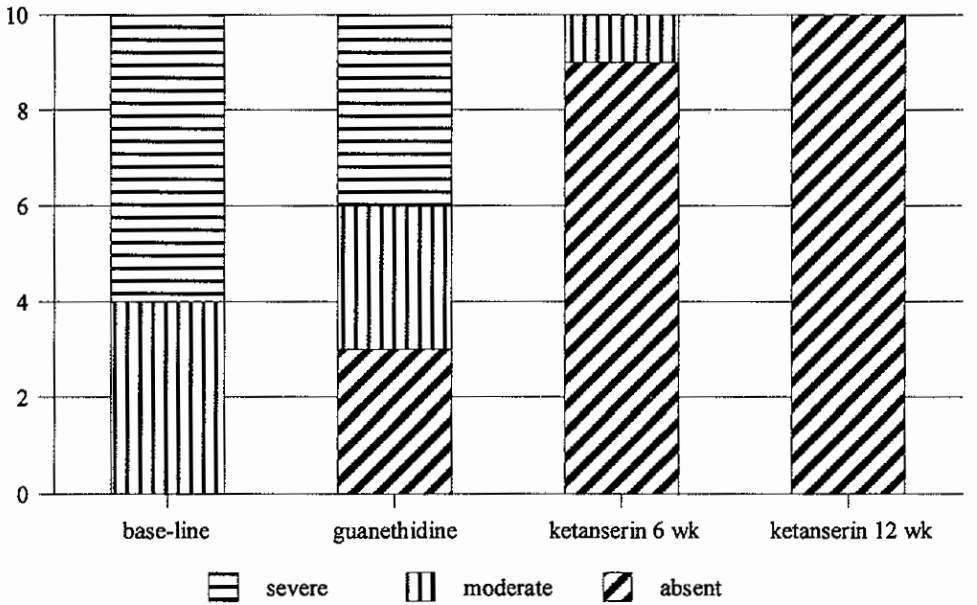

Fig. 7. Incidence of changes in skin temperature (T): absent ($T > 30°C$), moderate ($27°C < T < 30°C$), severe ($T < 27°C$).

Pain during exercise is shown in Fig. 8. At baseline, 2 patients had moderate pain during exercise and 8 severe pain. After guanethidine application, 8 had moderate complaints and 2 still had severe pain. In the evaluation after 6 weeks oral ketanserin medication, 5 patients were free of pain complaints during exercise and the remaining 5 still had moderate pain. At the end of the evaluation period all the patients were free of pain complaints during exercise. After this 12 week period of treatment, 5 patients were completely cured and could stop further medication. In the 5 remaining patients, function of the affected limb was normalised, but they preferred to continue ketanserin

treatment. The improvement in impaired mobility and the decrease of pain and of the increase of skin temperature were statistically significant ($p < 0.05$) after 6 and 12 weeks of ketanserin treatment compared with the results after the guanethidine treatment. No serious side-effects were observed in any patient.

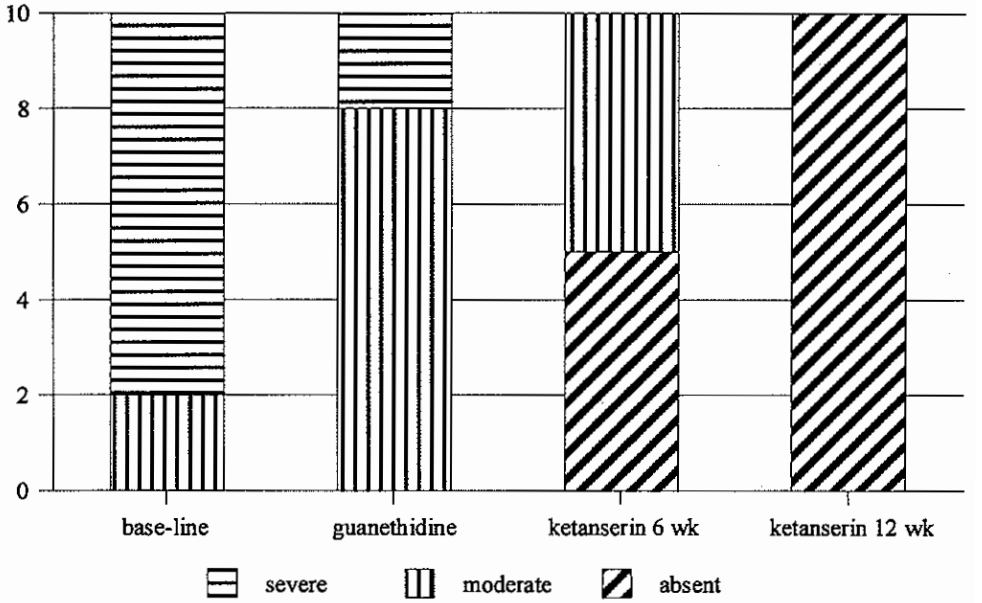

Fig. 8. Incidence of the symptom of pain during exercise, before treatment (baseline), after guanethidine treatment, and after 6 and 12 weeks of ketanserin therapy.

7.3 Pilot study with ketanserin in CRPS patients

After the previous encouraging results, CRPS patients were selected who were not treated with any therapy in the previous two months which could influence the sympathetic nervous system. This pilot study included 14 patients (mean age 33.9 years, mean duration of complaints 2.7 years). The diagnosis CRPS was made as previously described (section 7.1). First, a dosage of 10 mg ketanserin i.v. was given. If the skin temperature did not increase by at least 1°C after 30 minutes, another dosage of 10 mg i.v. was administered. In addition to skin temperature measurement (see before), continuous photo-plethysmography of the affected hand (digit 2) or foot (digit 1), blood pressure and heart rate were recorded. The patient data are summarised in Table IX.

Table IX. Patient data: intravenous ketanserin treatment group.

Number of patients	14
Male/Female	6/8
Age (years) mean ± SD	33.9 ± 2.4
Duration of complaints mean ± SD	2.7 ± 0.7
Site of complaints - leg	8
- arm	5
- both	1
Etiology - fracture	1
- contusion	5
- surgical wound	5
- other/unknown	3
Previous therapy not in the last two months - guanethidine	7
- others	4
- none	3

The mean interdigital temperature increased from 27.2 ± 0.6 to 29.0 ± 0.8 °C ($p < 0.001$) half an hour after the first administration of 10 mg ketanserin i.v. (Fig. 9). In 8 patients the skin temperature increased more than 1°C, from 27.9 ± 1.0°C to 33.5 ± 0.4. The remaining 6 patients received a second dose of 10 mg ketanserin i.v. after which the mean temperature increased more than 1°C, from 26.0 ± 0.4°C to 29.4 ± 1.3. The first administration of ketanserin resulted in a widening of the amplitude of the photo-electric plethysmogram to an average of 225% after 30 minutes ($p > 0.01$) (Fig.10). In patients who received a second dose, this gave rise to a widening to 168% after 30 minutes, compared to the amplitude before the second dose.

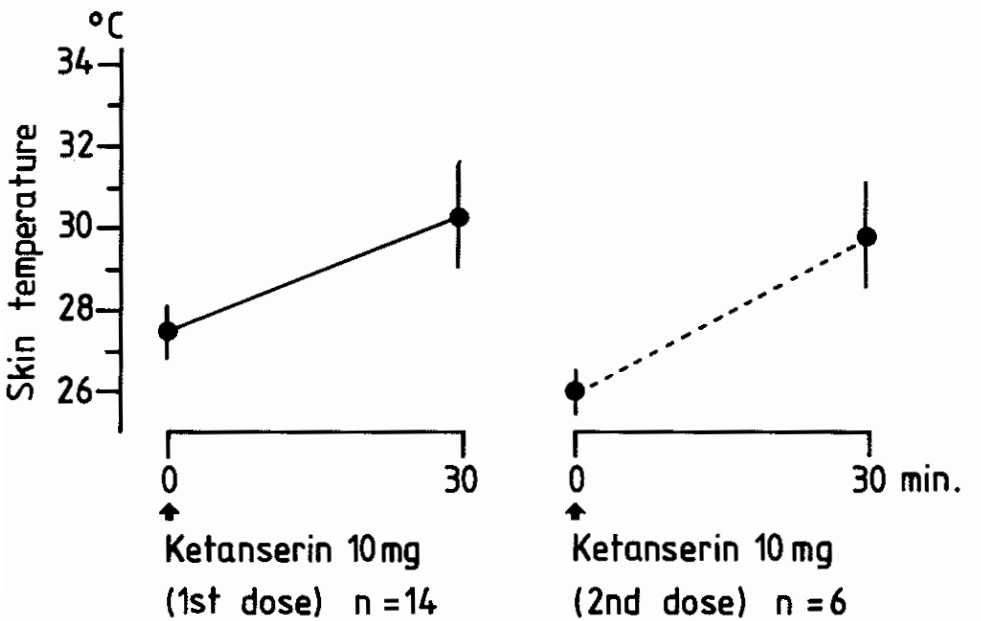

Fig. 9. Effects of ketanserin 10 mg i.v. on skin temperature. Values are mean ± SD.

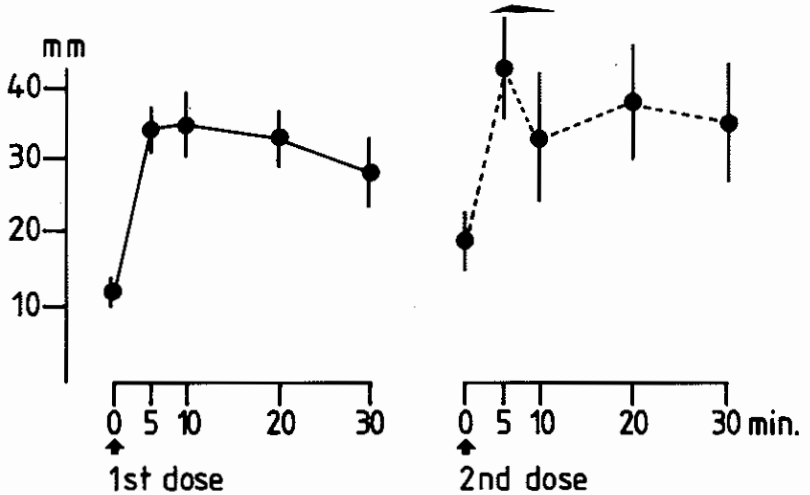

Fig. 10. Effects of ketanserin 10 mg i.v. on plethysmographic amplitude (in mm). Values are mean ± SD. Solid line, 1st medication n=14, mean at baseline 12.0 ± 2.3, after 30 min 27.0 ± 12.3. Dashed line, 2nd medication n=6, mean at baseline 19.0 ± 3.1, after 30 min 32.0 ± 7.1.

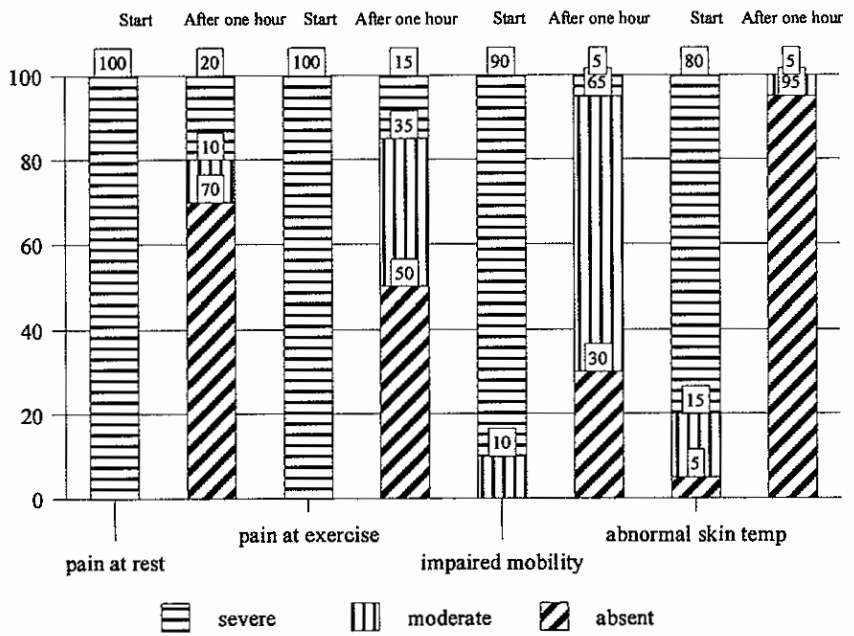

Fig. 11. Symptom score one hour after 10 mg ketanserin i.v. in the pilot study group of CRPS patients.

One hour after i.v. treatment the patients were scored for the symptoms, pain at rest, pain during exercise, impaired mobility and abnormal skin temperature in the affected extremity (Fig.11). It was noteworthy that the change in skin temperature of the affected

extremity was as dramatic as the change in the symptom pain at rest. This confirmed the initial impression we had in the previous study. After the i.v. loading dose of ketanserin these patients received oral maintenance therapy of ketanserin (daily dosage 60 – 120 mg) and were included in the follow-up study (see section 7.5).

Three patients experienced transient lightheadedness shortly after ketanserin was administered. None of the patients showed a clinically important decrease in blood pressure or increase in heart rate (Fig.12).

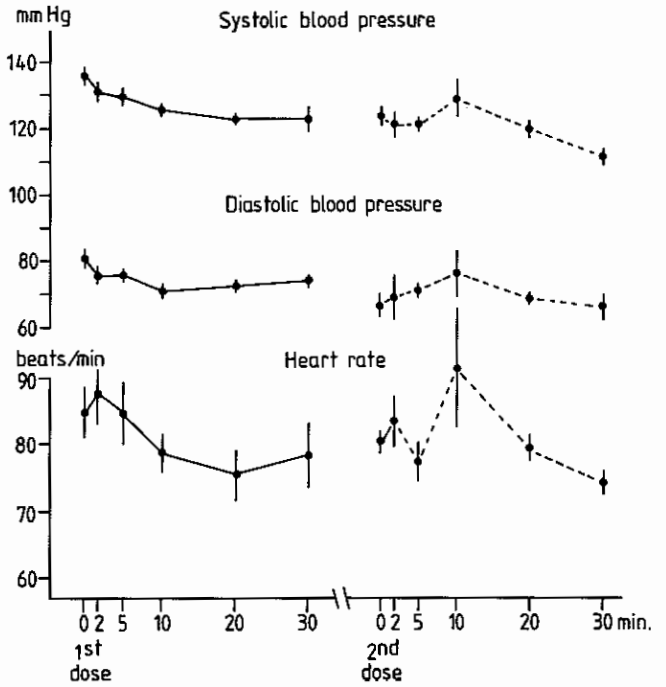

Fig. 12. Data on hemodynamic parameters of the 10 patients in the pilot study, after the first and second dose of ketanserin. Hemodynamic parameters after the first ketanserin dose; systolic blood pressure, n=14, mean ± SD at baseline 137.9 ± 3.9 mm Hg, after 30 min 121.3 ± 3.1 mm Hg. Diastolic blood pressure at baseline 82.3 ± 3.7 mm Hg, after 30 min 76.3 ± 2.0 mm Hg. HR at baseline 82.3 ± 6.9 beats/min, after 30 min 76.9 ± 7.0 beats/min.

Hemodynamic parameters after the second dose ketanserin; systolic blood pressure, n=6, mean ± SD at baseline 133.8 ± 5.1 mm Hg, after 30 min 131.3 ± 10.5 mm Hg. Diastolic blood pressure at baseline 78.7 ± 4.6 mm Hg, after 30 min 73.3 ± 2.7 mm Hg. HR at baseline 88.5 ± 1.7 beats/min, after 30 min 84.7 ± 2.9 beats/min.

7.4 Double-blind cross-over study with ketanserin in CRPS patients

A double-blind cross-over study was performed in which ketanserin or placebo was administered to 16 patients; their data are given in Table X. The patients were diagnosed and treated as the previous group.

Table X. Patient data: double-blind cross-over study group.

Number of patients	16
Male/Female	5/11
Age (years) mean ± SD	56.4 ± 7.6
Duration of complaints (years) mean ± SD	8.1 ± 2.4
Site of complaints - leg	5
- arm	9
- both	2
Etiology - fracture	3
- contusion	2
- surgical/wound	2
- ther/unknown	9
Previous therapy not in the last 2 months - guanethidine	4
- others	10
- none	2

In this study one group of 7 patients was treated with ketanserin 10 mg. i.v. followed by placebo i.v. (K - P), and one group of 9 patients with placebo i.v. followed by ketanserin 10 mg i.v. (P - K), with an interval of 30 minutes between the administration of ketanserin and placebo. For differences between the two treatment groups the Mann-Witney U-test with two-tailed probability was used. Differences were considered significant at a p-value < 0.05.

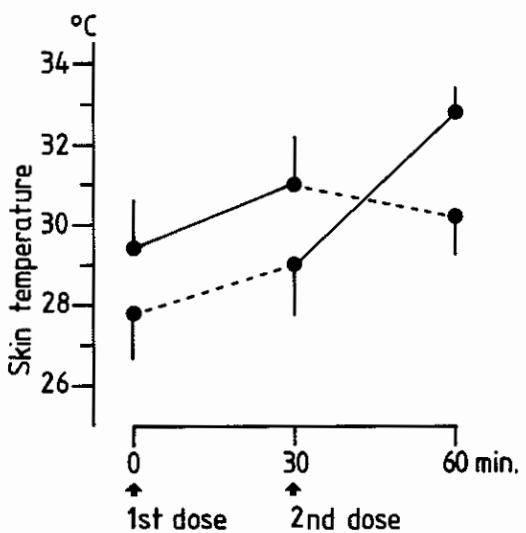

Fig. 13. Effects of ketanserin and placebo on skin temperature. Solid line for ketanserin 10 mg i.v., dashed line for placebo i.v., values are mean ± SD.

The first administration resulted in a significant increase in skin temperature after 30 minutes (Fig.13) both for ketanserin (29.5 to 31.4°C, $p = 0.03$) and placebo (26.8 to 28.7°C, $p < 0.001$) groups. The difference between the groups was not significant. Ketanserin administration following placebo resulted in a marked and significant increase in skin temperature (28.7 to 32.9°C, $p < 0.001$), whereas skin temperature decreased after placebo following ketanserin (31.4 to 30.3°C, $p > 0.05$); this difference was not significant. After the second administration the difference was significantly in favour of ketanserin ($p < 0.01$). The amplitude of the photo-plethysmogram showed significant increases 30 minutes after ketanserin ($p = 0.03$ in the K-P group, $p = 0.02$ in the P-K group) but not after placebo (Fig.14). The differences between ketanserin and placebo were significantly in favour of ketanserin ($p < 0.01$ for both the first and second administration).

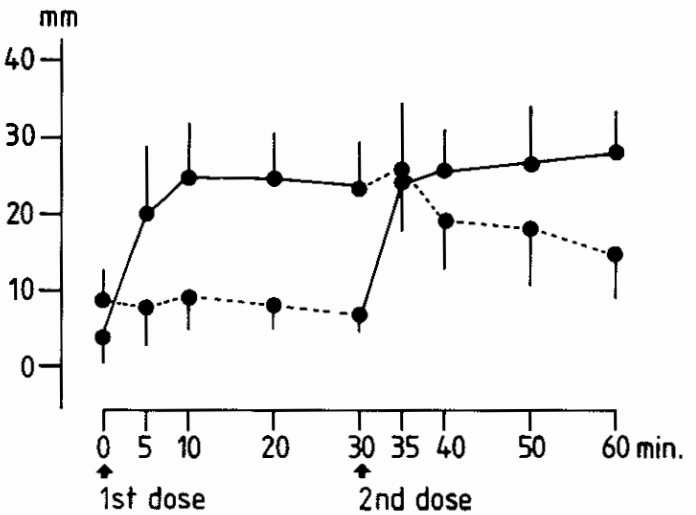

Fig. 14. Effects of ketanserin and placebo on plethysmographic amplitude, solid line for ketanserin 10 mg i.v., dashed line for placebo i.v., values are mean ± SD.

The effects of ketanserin and placebo on subjective symptoms are shown in Fig.15. Pain at rest in sequence ketanserin-placebo (n=7) gave a mean score at start of 1.9±0.4, after ketanserin i.v. 0.9±0.3, and after the placebo injection 1.0±0.4. In the sequence placebo-ketanserin (n=9) the values were: start, 1.7±0.4, after placebo 1.3±0.4, and after ketanserin 0.8±0.3. So there were no significant differences (p=0.20).

The values for impaired mobility in the ketanserin-placebo group were at start 1.7±0.5, after ketanserin 1.2±0.5, and after placebo 1.2±0.5, and in the placebo-ketanserin group the values were at start 1.3±0.3, after placebo 1.4±0.4, and after ketanserin 0.9±0.3; so there were no significant differences (p=0.06)

In case of pain during exercise there was a significant difference (p=0.05). In the sequence ketanserin-placebo the values were: start 2.1±0.3, after ketanserin 1.4±0.3, and after placebo 1.3.±0.4. In the sequence placebo-ketanserin the values were: start 2.6±0.2, after placebo 2.2±0.2, and after ketanserin 1.0±0.4.

For the symptom feeling of cold extremity we also found a significant difference (p=0.02): for the sequence ketanserin-placebo the start values were 1.6±0.4, after ketanserin 0.6±0.3, and after placebo 0.6±0.2. In the sequence placebo-ketanserin the values were: start 2.2±0.2, after placebo 1.7±0.3, and after ketanserin 0.0±0.0. Three of the 16 patients experienced transient light headedness after i.v. administration of ketanserin. No such effects were seen after placebo.

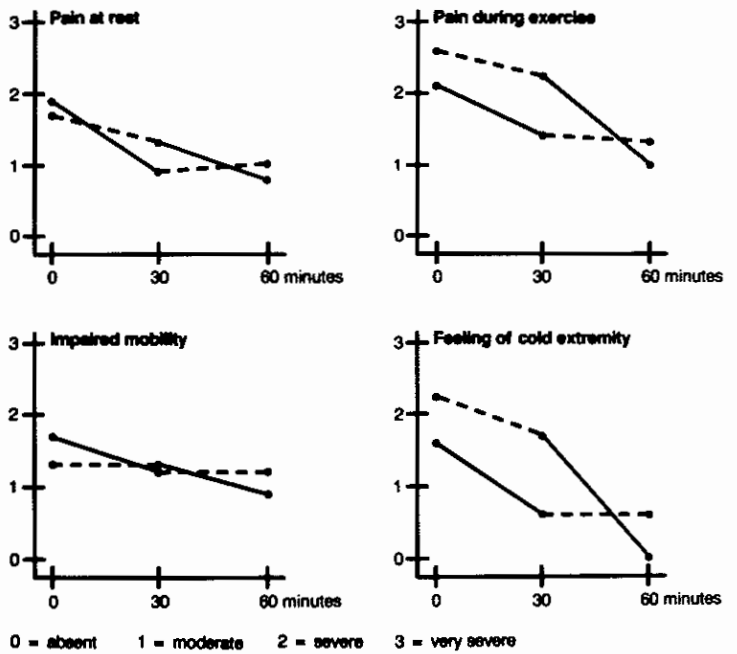

Fig. 15. Effects of ketanserin and placebo on the mean symptom scores, solid line for ketanserin 10 mg i.v., dashed line for placebo i.v.

In these 16 patients hemodynamic changes were also recorded. Again, no serious clinical changes were measured (Fig.16). From this double-blind cross-over study we conclude that ketanserin has a positive effect on the peripheral affected circulation of CRPS patients. This indicates that serotonin plays a role in the pathogenesis of the CRPS. When we are able to antagonize this effect of serotonin we may be able to solve many of the problems associated with pain in CRPS patients.

7.5 Follow-up study during 6 months treatment of CRPS patients with ketanserin

In a follow-up study, use of ketanserin was investigated in patients suffering from CRPS. Over a period of three years, 45 patients were treated with oral ketanserin in three dosages per day (dose ranging from 80 to 120 mg daily).

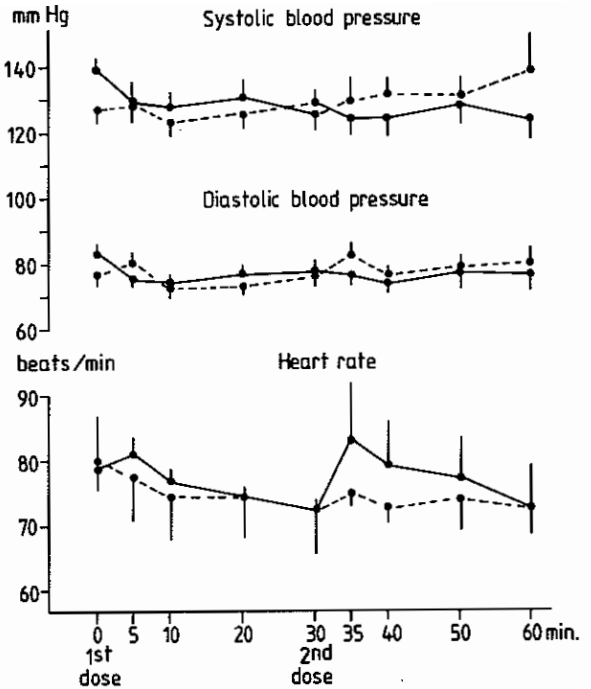

Fig. 16. Systolic/diastolic blood pressure and heart rate measurements during ketanserin or placebo administration, values are mean ± SD, solid line for ketanserin 10 mg i.v., dashed line for placebo i.v.

Table XI. Patient data follow-up study group.

Number of patients	45
Male/Female	16/29
Age (years) - mean ± SD	44.6 ± 12.8
- range	16–60
Duration of complaints (months) - mean ± SD	23.9 ± 8.6
- range	1–168
Etiology - fracture	20 (44%)
- contusion	17 (38%)
- others	8 (18%)
Previous therapy not in the last two months - guanethidine	7
- physiotherapy	8
- others	4
- none	26

Patient data are shown in Table XI. Of these 45 patients, a subgroup of 11 patients received i.v. ketanserin prior to the oral maintenance therapy. The rationale for this was to confirm at the beginning of the study that ketanserin did indeed improve circulation of the affected limb of CRPS patients. Later, we found that this pretreatment was of clinical interest.

The diagnosis of CRPS was based on two criteria. The first was that at least four of the CRPS symptoms were present (see section 7.1). The second was the occurrence of a trauma (not necessarily a fracture), followed by development of CRPS. Patients were

excluded if they had guanethidine treatment within the previous 2 months, sympathetic blockades, or any other treatment which can influence the sympathetic nervous system. Presence or absence of the CRPS symptoms was scored at 3 and 6 months, and the presence or absence of the symptoms were scored. Four classes of clinical results were scored: 1) severe; no change in symptoms, 2) moderate; presence of two or more symptoms and the presence of pain at rest, 3) mild; pain only during exercise, 4) good; no symptoms and absence of pain.

The main etiological factors were fracture and contusion (n=37). The other patients were operated on the affected extremity (n=6), one developed an ulcer after an infusion, and one patient had a local infection of unknown origin (Table XI). Figure 17 shows the number of patients and the number of their symptoms at the start of the study, and after 3 and 6 months.

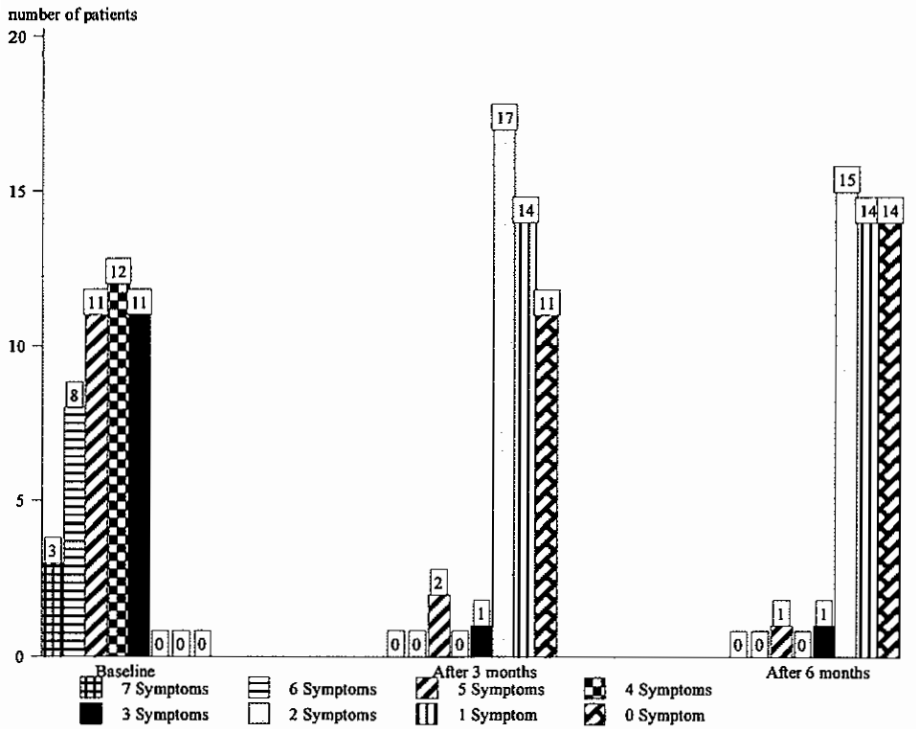

Fig. 17. Total number of symptoms scored in the 45 CRPS patients.

There is a clear decrease in the number of symptoms with time. After 3 months, 11 of the 45 patients were free of disabling CRPS symptoms, and after 6 months 26 patients (60%) were symptom free. At 6 months, 14 of these 26 patients had stopped the ketanserin medication for at least one month, with no relapse of symptoms. Figure 18 shows the incidence of the CRPS symptoms in the patients.

Clearly, the four most important symptoms of CRPS are abnormal skin temperature, pain at rest, pain on movement, and impaired mobility. The three latter symptoms have the most disabling effect on patients with CRPS, especially when the character of pain

is complicated by hyperpathia and/or allodynia. Surprisingly, the symptom of abnormal skin temperature, which runs parallel with the symptom of pain at rest, shows the greatest change when scoring after 3 months. Thus, when we succeed in restoring the circulation in such a way that the skin temperature normalises, the pain at rest may also cease. However, normalisation of skin temperature does not mean that the total circulation is normalised, because the symptom pain on movement persists longer and ceases at a slower rate. After 6 months, the symptom of pain on movement was still present in 40% of the patients, while the symptoms of abnormal skin temperature, hyperhidrosis, and edema were present in only 11% of the patients.

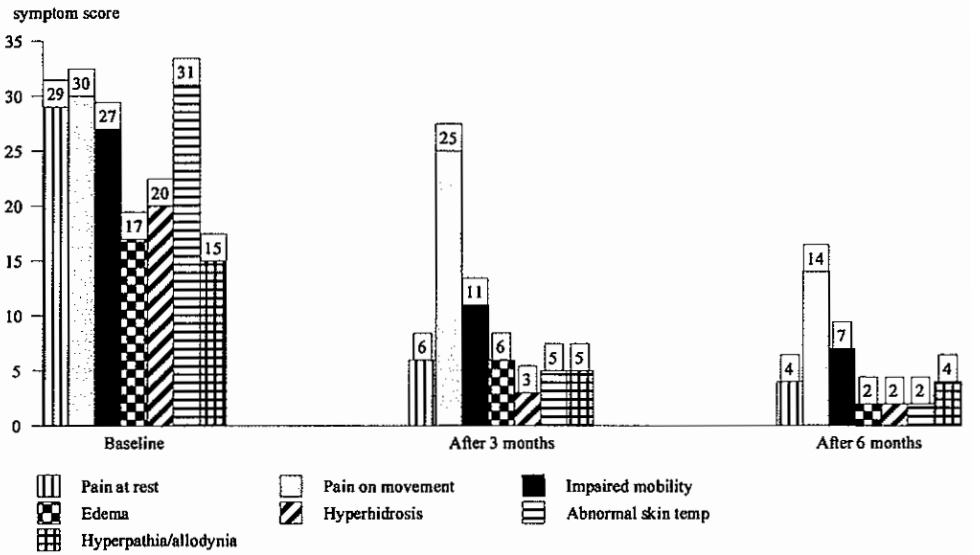

Fig. 18. Trends in symptomatology in the 45 CRPS patients.

In case of several modes of treatment in the literature, an important factor influencing the degree of success was found to be the length of time the patient had suffered from CRPS. When the condition has persisted for more than one year, it proved difficult to achieve a satisfactory result without remaining complaints. Thus, the time factor appeared to be very important for successful treatment of CRPS patients. This is supported by the fact that many studies on CRPS treatment, reported results on complaints lasting only for a few months. We divided 42 CRPS patients into two sub-groups: one group having symptoms for less than one year, and the other group with symptoms for more than one year (Fig.19). Three patients were unable to specify the exact duration of their complaints and were excluded from this part of the study. Statistical analysis (Mann-Whitney test: $p > 0.05$; two tailed) showed no significant differences between these two groups with regard to reduction in complaints. This is not in agreement with other studies reporting that the overall condition of patients with CRPS persisting for more than one year is more seriously debilitating.

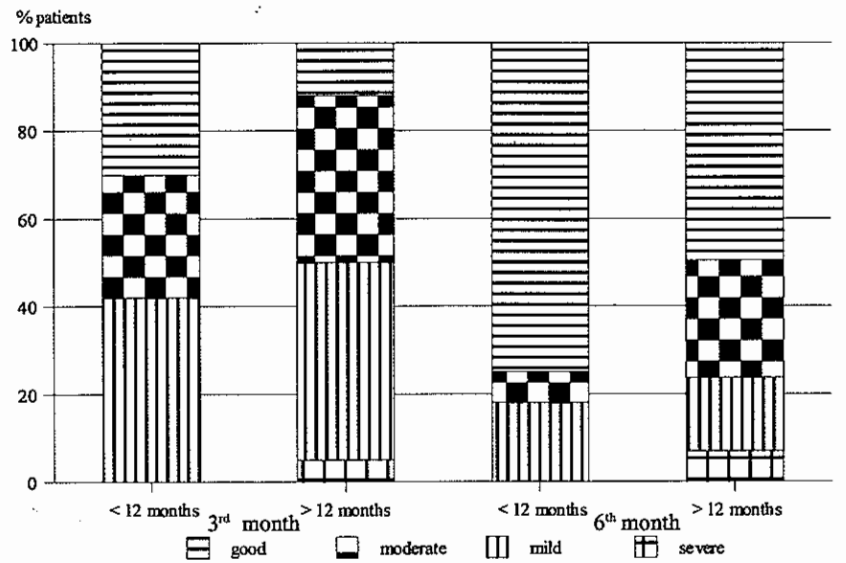

Fig. 19. Clinical results of 42 CRPS patients categorised by duration of complaints.

After testing many CRPS patients with i.v. ketanserin to establish whether this had any effect on the affected limb, we learned that pretreatment of the CRPS patient with i.v. ketanserin before oral daily intake of ketanserin, has a beneficial effect on the degree of reduction in CRPS complaints. Figure 20 shows the results of these two sub-groups of patients at 3 and 6 months. The subgroup receiving an intravenous starting dose of ketanserin showed the best therapeutic results. After 6 months 9 of the 11 patients were free of CRPS symptoms. Of the 34 patients not receiving an intravenous starting dose, 18 patients were symptom free after 6 months. Although the difference in clinical results seems obvious, analysis (Mann-Whitney test $p > 0.05$; two tailed) showed no statistical significant difference.

Five patients reported dizziness as side-effect of the treatment. In one patient this led to cessation of ketanserin treatment after 3 months. This was not problematic because all 5 patients were free of CRPS symptoms and complaints. Two of the patients received a lower dose of ketanserin, after which the dizziness disappeared. Two other patients had tachycardia. In one the ketanserin medication was stopped after 3 months because the patient was symptom free, in the other a lower dose resulted in cessation of tachycardia. No other side-effects were reported during the treatment of these 45 patients.

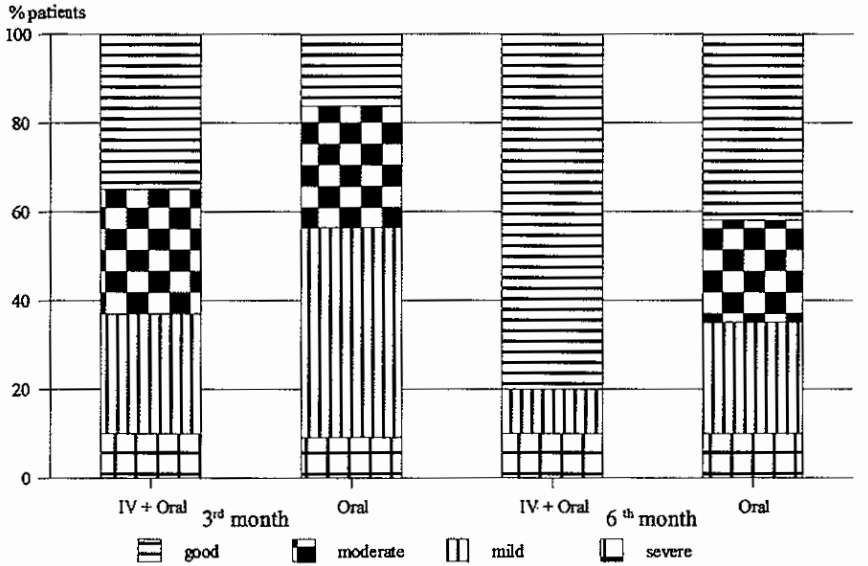

Fig. 20. Clinical results of the 45 patients divided into i.v. ketanserin plus oral treatment and oral treatment alone.

In conclusion, ketanserin therapy is very effective in patients suffering from CRPS. An initial intravenous loading dose seems to be preferential in the effort to restore the balance between vasoconstriction and vasodilatation. An oral maintenance dose reduces the symptoms in at least 60% of the patients. The generally accepted view that CRPS persisting for more than one year cannot be successfully treated is not true for treatment with ketanserin.

Chapter 8 BIOCHEMICAL FUNCTION AND PHARMACOLOGY OF CARNITINE

8.1 Carnitine

$(CH_3)_3N^+ - CH_2 - CHOH - CH_2 - COO^-$

Fig. 21. Structure of carnitine (β-hydroxy-γ-trimethylammonium butyrate).

Carnitine (Fig. 21) is present in all mammalian tissues (Bremer 1984). It was discovered by Krimberg in 1905 in a bovine muscle extract. In 1952 Carter et al. found that carnitine was a growth factor for the larvae of the meal worm Tenebrio molitor. Friedman and Fraenkel established in 1955 that carnitine was reversibly acetylated by acetyl-CoA, and Fritz (1955) discovered that it stimulated long-chain fatty acid oxidation in liver homogenates and mitochondria. Later studies (Bremer 1963, Fritz et al. 1963, Pande 1975, Ramsay and Tubbs 1995) revealed that carnitine acts as a carrier for activated fatty acids across the inner-mitochondrial membrane, and that the acyl-group is exchanged between carnitine and CoA through the action of carnitine acyltransferases, and that acylcarnitine is transported over the mitochondrial inner membrane.

Carnitine exists in the form of two stereoisomers (enantiomeres). Only the L-form is active as acyl carrier. Esters can be formed between the carboxyl group of carnitine and an alcohol, or between the alcohol group of carnitine and an acid. The naturally occurring esters, acetyl-L-carnitine, propionyl-L-carnitine and the acylcarnitines with a longer fatty acid chain, belong to the second category. In recent years, acetyl-L-carnitine and propionyl-L-carnitine, became available and were tested for their efficacy in animal models and human diseases. Acetyl-L-carnitine was tested in Alzheimer disease (Spagnoli et al. 1991, Pettegrew et al. 1995) and propionyl-L-carnitine in cardiovascular diseases (De Jong and Ferrari 1995, Bartels 1996, Anand et al. 1998).

8.2 Role of carnitine in mitochondrial metabolism

As mentioned above, one of the first recognised functions of L-carnitine was the transport of activated long-chain fatty acids over the mitochondrial inner membrane into the matrix. Long-chain fatty acids are activated into their CoA-esters on various membranes of the cell; in mitochondria on the outer face of the mitochondrial outer membrane (De Jong and Hülsmann 1970; Pande and Blanchaer 1970). Because long-chain acyl-CoA cannot pass through the mitochondrial inner membrane, to be oxidised in the matrix, it is converted into acylcarnitine. This reaction is catalysed by carnitine palmitoyltransferase I (CPT I), located in the mitochondrial outer membrane (Murthy and Pande 1987). The activity of this enzyme is controlled by its inhibitor malonyl-CoA (McGarry and Foster 1983). The import of acylcarnitine over the inner membrane is catalysed by carnitine-acylcarnitine translocase. This transport enzyme catalyses the exchange

between acylcarnitine from the intermembrane space and carnitine from the matrix (Pande 1975; Ramsay and Tubbs 1975), and when needed between (acyl)carnitine and (acyl)carnitine, while it also catalyses the unidirectional import of carnitine (Pande and Parvin 1980). In the matrix, acylcarnitine reacts with CoA to form acyl-CoA and carnitine. This reaction is catalysed by carnitine palmitoyltransferase II (CPT II), which is attached to the inside of the mitochondrial inner membrane. Now the enzymes of the mitochondrial beta-oxidation can degrade the acyl-CoA. This series of events is schematically shown in Fig. 22.

A third transferase, carnitine acetyltransferase, is also attached to the inside of the mitochondrial inner membrane and catalyses a similar reaction as CPT II, but with shorter acyl groups. Carnitine acetyltransferase is involved in import and export of acetyl groups and short and medium-chain acyl groups, it buffers the mitochondrial acetyl content and releases CoA from acetyl-CoA, which stimulates pyruvate dehydrogenase (Uziel et al. 1988; Scholte et al. 1996). Consequences of stimulation of the pyruvate oxidation by carnitine are a decrease in the production of lactic acid (Ferrari and Visioli 1995), and stimulation of glucose oxidation (Hoppel 1992; Lopaschuck and Gamble 1994). In isolated rat heart, it was shown that the product of pyruvate oxidation, acetyl-CoA, is exported from the mitochondria as acetylcarnitine and is then carboxylated to malonyl-CoA, the inhibitor of CPT I, which causes a decrease in mitochondrial long-chain fatty acid oxidation (Lopaschuck and Gamble 1994).

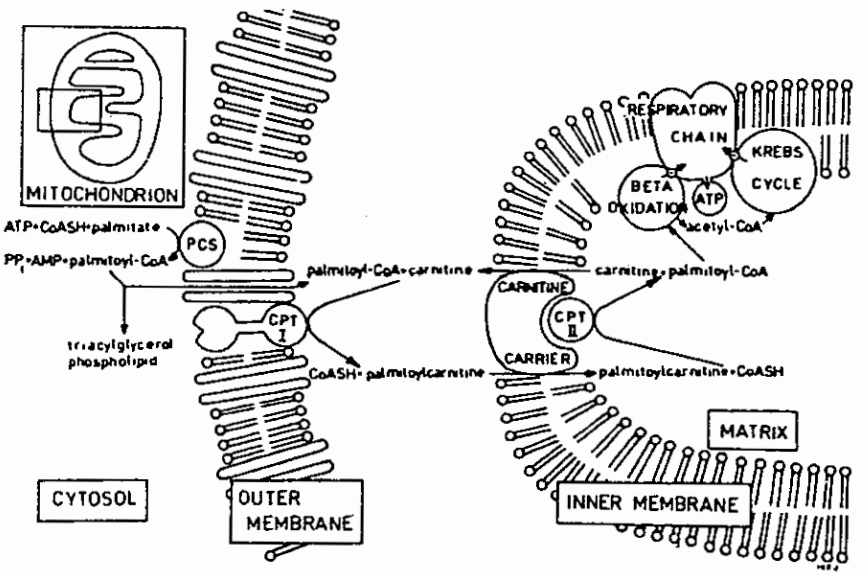

Fig. 22. Fatty acid catabolism by mitochondria. PCS = palmitoyl-CoA synthetase, CPT = carnitine palmitoyltransferase, ATP = ATP synthetase. The catalytic site of CPT I is on the inner face of the outer mitochondrial membrane, while the malonyl-CoA binding site of the regulatory subunit is on the outer face of the membrane (Scholte and Jennekes 1988).

Carnitine acetyltransferase also releases CoA from higher acyl-CoA esters and promotes 2-ketoglutarate dehydrogenase and thereby the Krebs cycle (Hülsmann et al. 1964) and branched-chain amino acid metabolism (Wagenmakers 1984).

Acetylcarnitine is the most important acylcarnitine species in blood. It can be oxidised by all mitochondria, including those from cells such as neurons and granulocytes that lack long-chain fatty acid oxidation (Scholte and De Jonge 1987). It is important as end product of the beta-oxidation in peroxisomes. Acetylcarnitine is an inhibitor of apoptosis (Galli and Fratelli 1993, Cossarizza et al. 1997). It is likely that the carnitine system in brain is separated from the circulation by the blood-brain barrier. This is indicated by the fact that the brain can synthesise its own carnitine. Kuratsune et al. (1997a) reported that the acetyl moiety of acetylcarnitine is able to pass the blood-brain barrier and is important for brain metabolism. Pharmaceutical effects of acetylcarnitine on the central nervous system have been reported by Kuratsune et al. (1997b).

Kuratsune et al. (1994) and Plioplys and Plioplys (1995) showed that patients with chronic fatigue syndrome had a deficiency in plasma acylcarnitine. As there are persons with (very) low acylcarnitine levels and no fatigue, Scholte et al. (1998) hypothesised that low acylcarnitine is a risk factor for getting the condition.

The carnitine system functions also in the detoxification of acyl-CoA esters. Accumulation of these esters inhibits many enzymes. The inhibitory actions increase with the chain length of the acyl-moiety. Long-chain acyl-CoA is a potent inhibitor of the adenine nucleotide translocator, carnitine acetyltransferase and glutamate dehydrogenase (for a summary and references see Scholte et al. 1990). Deficiency of the adenine nucleotide translocator causes an increase in mitochondrial free radical production (Bakker et al. 1993a,b). Long-chain acyl-CoA is also one of the compounds that can destroy the mitochondria, by opening of the mitochondrial permeability transition pore (Pastorino et al. 1993). This event will give rise to a burst in free radical production. When the acyl-CoAs are oxidised by peroxisomes, one of the products is H_2O_2. So when long-chain acyl-CoAs are converted into acylcarnitines, this will prevent the production of free radicals.

The carnitine system is also able to detoxify acylcarnitine esters. In general, acylcarnitine is less toxic than the corresponding acyl-CoA, but its actions are different. Long-chain acylcarnitine (LCAC) interferes with signal transduction and metabolism in patients with severe deficiency of CPT II (Demaugre et al. 1991) or carnitine-acylcarnitine translocase (Pande and Murthy 1994). The accumulation of LCAC causes muscle weakness and rhythm disturbances of the heart, possibly by causing Caz^{2+} and Na^{+} membrane permeability (reviewed by Lamers 1995). LCAC inhibits the high affinity carnitine importer in the plasma membrane (for refs. see section 8.3).

Patients with inborn errors in mitochondrial acyl-CoA metabolism have a carnitine deficiency (reviewed by Scholte and De Jong 1987; Famularo et al. 1997) and treatment with carnitine promotes the conversion of the acyl-CoA into acylcarnitine and the release of acylcarnitine into the blood and urine. This process involves export of the acylcarnitine species from the mitochondria, followed by the efflux of acylcarnitine from the cell (see also section 8.4). It is not known if the latter process is catalysed by the high affinity carnitine importer in the plasma membrane (see section 8.3), or by another transport protein. In the kidneys carnitine is reabsorbed with high efficiency, while acylcarnitine

is less efficiently reabsorbed and partly lost with the urine (Carroll et al. 1981; Engel et al. 1981).

8.3 Carnitine transport over the plasma membrane

Carnitine is imported in many cells by the high affinity carnitine importer. The importer is stimulated by an inwardly directed gradient of Na^+ and Cl^- ions (De Jonge et al. in Scholte and de Jonge, 1987; Stieger et al. 1995). The importer is inhibited by valproate (Tein et al. 1993), and also by LCAC (Mölstad et al. 1977; Stanley et al. 1991; Scholte et al. 1996).

In primary systemic carnitine deficiency, the carnitine importer is defect. In controls the importer was present in the brush border membranes of kidney and the small intestine, and in the plasma membranes of heart, muscle and fibroblasts (Scholte et al. 1990, Tein et al. 1990, for reviews and refs.). With failure of the transporter, carnitine is not reabsorbed from the urine and excreted in high amounts. Carnitine is not available in food, and is excreted with the faeces; these losses cause very low plasma carnitine levels. Most patients present young with cardiomyopathy and are completely cured by carnitine treatment. Due to the lack of this type of treatment in the past, all such patients died. Because heterozygotes have half normal plasma carnitine levels and half normal carnitine import in cultured fibroblasts, it is likely that the activity of the carnitine importer determines the plasma carnitine levels. The precise plasma level in an individual is determined by the difference between input (renal reabsorption, intestinal transport, renal and hepatic biosynthesis) and output (uptake by muscle, heart and other cells) (Scholte et al. 1996).

In 1998 the human high affinity carnitine importer gene *OCTN2* was identified and characterised (Tamai et al. 1998), and the first mutations were described in patients with primary systemic carnitine deficiency (Lamhonwah and Tein 1998; Nezu et al. 1999; Tang et al. 1999; Wang et al. 1999; Vaz et al. 1999).

8.4 Other actions of carnitine with relevance to CRPS

Hülsmann and co-workers (1985, 1994, 1996, 1997) revealed the important role of the endothelial cells and the interstitium to explain the cardioprotective action of carnitine. This theory provides also an explanation for the therapeutic action of carnitine in circulation disorders such as peripheral arterial disease or claudicatio intermittence (summarised by Hiatt and Brass 1995), and in CRPS.

Ischemia due to capillary leakage (Hülsmann 1997) causes calcium overload and edema, which affects the microcirculation. Edema is swelling of the tissue due to an increase in the interstitial space between the endothelial cells, such as the (cardio) myocytes and the vascular cells. Tissue oxygenation decreases, which leads to an accumulation of long-chain acyl-CoA. This is converted into LCAC in the anoxic (cardio) myocytes. Hülsmann et al. (1996) observed in perfused rat hearts with insufficient oxygen supply a loss of interstitial compounds, including carnitine. There was also an increase of the catabolic products, lactate, urate and lysophosphatidylcholine. Carnitine

treatment restored flow and metabolism. These results were explained as follows: addition of carnitine results in repletion of interstitial carnitine, which exchanges with LCAC from various cells types. Reperfusion with low amounts of LCAC (around 1 μM) were previously found to result in the recovery of ischemic rat hearts (Hülsmann et al. 1985). By competition for negatively charged phospholipids for excessive binding of Ca^{2+} to the cell surface, resulting in avoidance of calcium overload of cells, which results in restoration of cell function (Hülsmann et al. 1996). This abolished endothelial Ca^{2+} overload, normalised the interstitial volume and restored the tissue perfusion and the aerobic metabolism in the (cardio) myocytes. Other amphiphilic cationic drugs acted likewise, but LCAC has the advantage over them that it is formed at the right time and place. Hülsmann et al. (1996) showed that the remaining LCAC was released to the blood in exchange for plasma carnitine, and demonstrated also a carnitine-induced release of LCAC from cultured endothelial cells in the presence of oleate. As outlined above (section 8.2), the molecular identity of this carnitine-acylcarnitine exchange protein is not yet completely clarified.

Carnitine treatment causes an increase in interstitial carnitine, which exchanges with LCAC accumulated in the ischemic cells. To achieve this effect, the vascular obstruction must be removed first, restoring flow and allowing carnitine to reach the site of ischemia. It is likely that pre-treatment with ketanserin fulfills this condition. After a period of less than 10 minutes vasodilatation was achieved, but there was not yet any improvement in the hyperpathic/allodynic pain. From our own studies (Chapters 10, 11) we know that, after one hour, an injection of 1g carnitine leads to a nearly nine-fold increase of plasma free L-carnitine, but a much lower increase in the carnitine esters. In general, we found higher levels of free L-carnitine, acetyl-L-carnitine and propionyl-L-carnitine in CRPS patients compared with healthy volunteers (Chapter 11). Sassen et al. (1991) reported that for the heart propionyl-L-carnitine is responsible for the reflow. It is probable that in CRPS patients with already higher levels of free L-carnitine, acetyl-L-carnitine and propionyl-L-carnitine, an injection of L-carnitine will also facilitate a vasodilatatory effect. The observed vasodilatation, sometimes also causing a substantial increase in skin temperature, (seen on the electro-plethysmogram), must be an indirect effect of the injection of carnitine after pre-treatment by ketanserin.

In 1997 Capecchi et al. published their results of studies aimed to clarify the mechanism of action of carnitine (and its derivatives acetyl-L-carnitine and propionyl-L-carnitine) on plasma levels of adenosine, ATP and inosine. Their investigations were performed in patients suffering from peripheral arterial disease. They concluded that propionyl-L-carnitine and acetyl-L-carnitine increased the plasma levels of adenosine and ATP, whereas L-carnitine induced less relevant changes. This effect was seen after 10 minutes, increased during the infusion period of 30 minutes, and decreased after the infusion ended. The administration of the compounds did not affect the adenosine/inosine ratio. Peak plasma levels of adenosine preceded in any case those of ATP. This indicates that the pharmacological activity of propionyl-L-carnitine, acetyl-L-carnitine and L-carnitine may be mediated, at least in part, by an interference with the endogenous purine system. Normally, these are physiological mechanisms of tissue protection. Disturbance of these physiological protection mechanisms may play a role in peripheral circulatory diseases, in which we can probably also place the CRPS pathophysiology.

Another mechanism of action of L-carnitine, which may be therapeutic in CRPS,

has been proposed and summarised by Arduini et al. (1995). They showed that the carnitine system promotes membrane repair by reacylation of peroxidised fatty acyl groups in phospholipids. They stated that carnitine and carnitine palmitoyltransferase are involved in membrane phospholipid turnover in erythrocytes and neurons. Especially after ischemia and reperfusion, this mechanism can protect the tissue membranes from damage by free radicals. That carnitine esters appear to be more effective than carnitine, is not related to reacylation but to less understood effects, probably due to membrane stability (Koster 1995).

Based on the theoretical considerations presented in this chapter, and the positive clinical results found by van Oudheusden (personal communication 1996) in treating children with CRPS with carnitine and riboflavin, we tried to improve the ketanserin treatment of CRPS patients by the addition of carnitine.

Chapter 9 EFFECTS ON THE PHOTO-ELECTRIC PLETHYSMOGRAM, SKIN TEMPERATURE AND PULSE OXIMETRY TREND RECORDING IN CRPS PATIENTS TREATED WITH KETANSERIN AND CARNITINE

9.1 Effect of ketanserin on the photo-electric plethysmogram, skin temperature and pulse oximetry

In treating CRPS patients we experienced that treatment with an initial intravenous loading dose of 10 mg ketanserin, under controlled and monitored conditions, proved very effective. Figure 23 shows the effects of 10 mg i.v. ketanserin on the trend recording. These values were compared with those from 8 healthy female volunteers (controls). Results are given in Tables XII, XIII, and XIV.

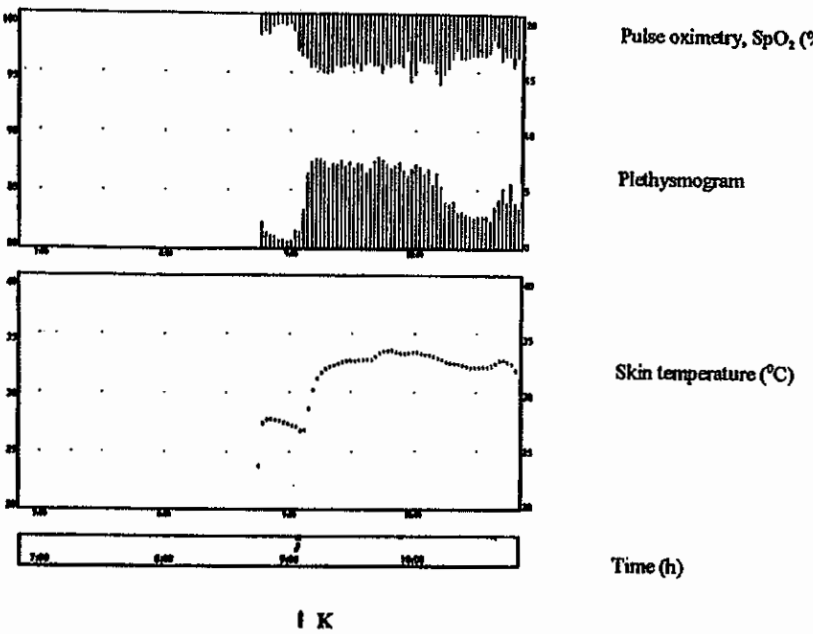

Fig. 23. Simultaneous trend recording of the effects of 10 mg i.v.ketanserin on skin temperature (lower part of registration), photo-electric plethysmography (middle part), and pulse oximetry (upper part) of the affected limb of a patient with CRPS. "K" indicates the moment of injection.

Table XII. Data on photo-electric plethysmographic trend recordings in 8 female CRPS patients compared with data from 8 controls. Measurements in mm at baseline and 1 hour after 10 mg i.v. ketanserin. Identical letters in superscript indicate significant differences by paired (a,b) and unpaired *t*-test (c).

	n	age, mean ± SD (years)	baseline mean ± SD	1 hour after 10 mg ketanserin i.v. mean ± SD	Δ change mean ± SD	max. Δ change mean ± SD
CRPS patients	8	41.3 ± 15.8	5.0 ± 1.9 [a]	15.4 ± 3.3 [a]	10.3 ± 3.5 [c]	15.7 ± 6.2
Controls	8	42.3 ± 15.3	3.1 ± 1.0 [b]	6.4 ± 1.6 [b]	3.3 ± 1.3 [c]	10.9 ± 6.7

Table XII shows that there is a greater widening of the plethysmogram after an injection of ketanserin in CRPS patients than in controls. We have already reported the beneficial effect seen on the plethysmogram after ketanserin in case of CRPS (Moesker et al. 1998c, Chapter 7). For statistical analyses the paired *t*-test was used. After ketanserin there was a significant widening of the plethysmogram in both the controls ($p<0.001$) and in the CRPS group ($p<0.001$).

In the CRPS group the average widening of the plethysmogram in Δ change was significantly greater than in controls. The average of max. Δ change was not significantly different.

Table XIII. Skin temperature measurements (in °C) in 8 CRPS patients compared with 8 controls at baseline and 1 hour after 10 mg i.v. ketanserin.

	n	age, mean ± SD (years)	baseline mean ± SD	1 hour after 10 mg ketanserin i.v. mean ± SD	Δ change mean ± SD	max. Δ change mean ± SD
CRPS patients	8	41.3 ± 15.8	26.7 ± 1.8	32.4 ± 1.1	5.6 ± 2.4	7.2 ± 1.2
Controls	8	42.3 ± 15.3	29.0 ± 1.3	29.8 ± 1.6	0.8 ± 0.9	2.3 ± 3.1

The change in skin temperature after i.v. ketanserin is also impressive. As shown in Table XIII, not only is the baseline skin temperature of the CRPS patients lower than in controls, but the rise in skin temperature and the maximal rise are significantly higher than in the controls. As reported earlier, these changes in skin temperature of the affected limb of CRPS patients have been proven statistically significant in a double-blind cross-over study (Chapter 7). Analyses with the paired *t*-test showed that in controls there was no significant change after ketanserin i.v.($p>0.05$), whereas in the CRPS patients there was a significant rise in skin temperature of 5.6 °C ($p<0.001$).

Table XIV. Values of SpO_2 in % in the pulse oximetry trend recording in 8 CRPS patients compared with 8 controls at baseline and 1 hour after 10 mg i.v. ketanserin.

	n	age, mean ± SD (years)	baseline mean ± SD	1 hour after 10 mg ketanserin i.v. mean ± SD	Δ change mean ± SD	max. Δ change mean ± SD
CRPS patients	8	41.3 ± 15.8	98.0 ± 0.8	96.4 ± 0.5	- 1.6 ± 0.9	- 2.8 ± 0.7
Controls	8	42.3 ± 15.3	98.6 ± 0.7	98.0 ± 1.1	- 0.6 ± 0.5	- 2.0 ± 1.2

The SpO_2 recordings of all CRPS patients showed a similar reaction, namely a lowering of the oxygen saturation, to levels lower than in the controls. The difference between the controls and the patients was not so striking as seen in the plethysmography and skin temperature recordings. Analyses using the paired *t*-test showed that in the controls there was no significant change $p>0.01$, whereas in the CRPS patients there was a significant decrease in oxygen saturation ($p<0.001$).

Thus, in all three monitored items, plethysmography, skin temperature and pulse-oximetry, after ketanserin there was a statistically significant change in CRPS patients.

9.2 Effect of carnitine on the trend recording

In studies monitoring plethysmographic changes using carnitine alone, there was no acute effect on the circulation of the affected extremity. This was not surprising because carnitine is not known as an acute vasodilatating drug. Brevetti et al. (1992) reported the results of a double-blind, cross-over study, in which they compared the effects of a single dose i.v. propionyl-L-carnitine versus i.v. L-carnitine on the walking capacity in patients with peripheral vascular disease. Their results indicated that neither drug affected the blood velocity nor the blood flow rate in the ischemic leg, suggesting that the beneficial effect on the walking capacity was dependent on some metabolic action.

In 1996 Giamberardino et al. described the relation between muscle exercise, pain and metabolism. Eccentric muscle effort is known to induce delayed muscle soreness and muscle damage which are not responsive to medical treatment with the most common analgesic agents. They studied the effect of 3 weeks oral L-carnitine supplementation, and found that this treatment significantly reduced pain, tenderness and CK release after the eccentric effort compared to placebo. In contrast, no significant difference was found without medication. They concluded that L-carnitine has a protective effect against pain and damage from eccentric effort. This effect was mainly attributed by the authors to the vasodilatation property of the compound, which both improves energetic metabolism of the hypoxic/damaged muscle and enhances wash-out of algogenic metabolites.

The next step in the development of the treatment for CRPS patients was to infuse 1 g carnitine after the ketanserin; about 10 minutes after carnitine injection we observed an extra widening of the plethysmogram. These vasodilatatory effects may be the result of the widening of two different systems of the microcirculation. These two systems may also have different regulatory systems, as reported by Van Dielen et al. (1998). They studied the effect of surgical sympathectomy on skin blood flow in a rat model of chronic limb ischemia. They found indications that in case of lower limb ischemia sympathectomy, skin blood flow improves at the thermoregulatory but not on the nutritional level of skin microcirculation. This may be related to the fact that the thermoregulatory vessels are mainly sympathetically controlled, whereas the nutritional capillaries are mainly controlled by local (nonneural) factors.

We prefer to explain the result by assuming that ketanserin (probably due to vasodilatation) enables carnitine to reach its target organ, the endothelium, and we accordingly re-organised the intravenous treatment for CRPS patients. After treatment with 10 mg ketanserin by bolus injection which is followed by i.v. medication of 4 mg per hour, the carnitine infusion was started after one hour. This enabled us to measure the effect

of carnitine on the plethysmogram. Figure 24 shows the specific reproducible effect of carnitine after prior treatment of the vasoconstriction by ketanserin.

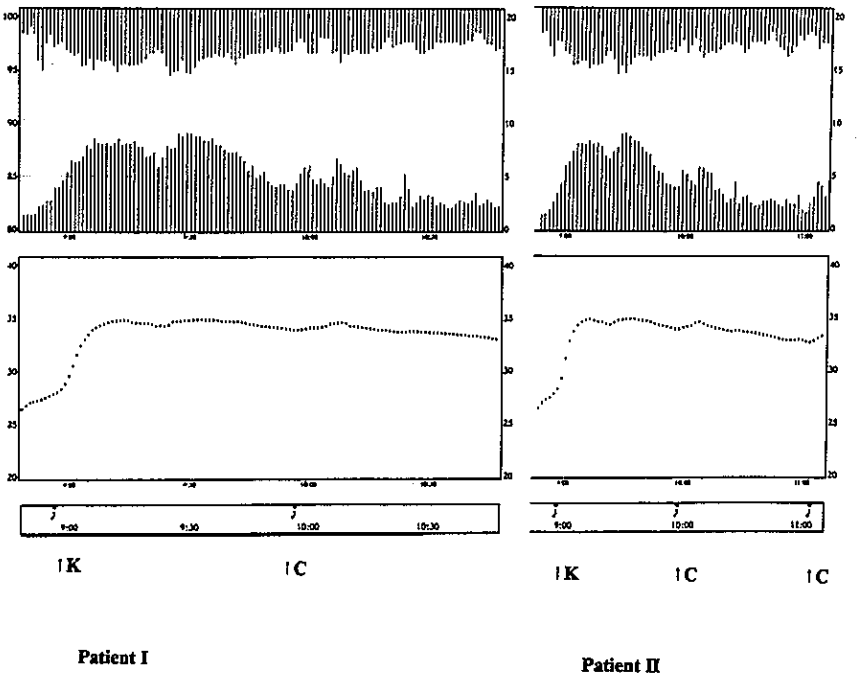

Fig. 24. Effect of 1 gram i.v. carnitine on the simultaneous trend recording of skin temperature monitoring, plethysmogram, and pulse oximetry after relieving vasoconstriction by 10 mg i.v. ketanserin and continuation of the ketanserin medication at a rate of 4 mg per hour. Both trend recordings, left and right, are examples of observations made in two different CRPS patients. K is injection of ketanserin, C is injection of carnitine. The lowest part shows skin temperature, the middle part the plethysmogram, and the upper part shows the oxygen saturation as monitored in trend recording (see also Fig. 23).

It is remarkable that at the start of treatment in most CRPS patients treated initially with ketanserin the carnitine i.v. injection caused an extra increase in skin temperature of 0.5°C and a simultaneous widening of the plethysmogram of 2 or 3 mm.

9.3 Effect of ketanserin and carnitine on the skin temperature, plethysmogram and pulse oximetry on the healthy extremity compared with the CRPS side

To obtain evidence that in the pathophysiology of CRPS not only a disregulation of peripheral mechanisms of the circulatory flow is involved, but also higher levels of regulatory systems, we measured as well the circulatory flow of the non-affected limb of CRPS patients. Figure 25 shows the simultaneous trend recordings of the affected CRPS side (left) and the contralateral healthy side (right).

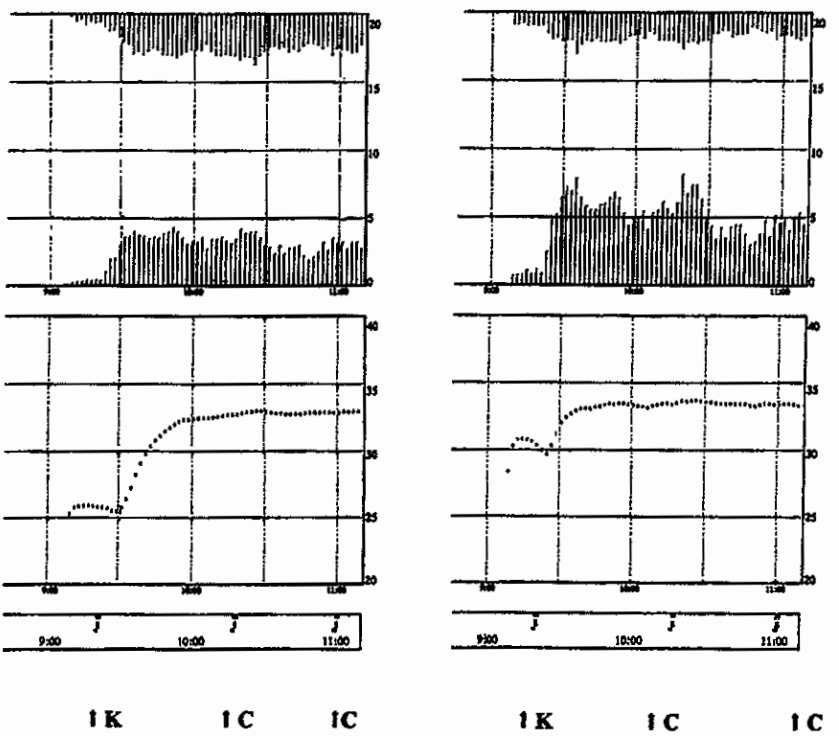

Fig. 25. Effect of ketanserin and carnitine on the trend recording of the skin temperature, plethysmogram, and pulse oximetry on the affected CRPS side (left) and on the contralateral healthy side (right) (see also legend of Fig. 24).

Although the baseline skin temperature of the non-affected side was higher than the affected side, also on the non-affected side a rise in skin temperature was observed after i.v. ketanserin. The same reactions were recorded with the plethysmogram and the pulse oximetry.

In 1989 Rosén et al. published results on the bilateral involvement and the effect of sympathetic blockade on skin microcirculation in CRPS. They concluded that there is a disturbance of a central nervous mechanism in patients with unilateral CRPS.

Kurvers et al. (1996a) showed also by means of contralateral observations a spinal component in CRPS. In the model of the loose sciatic nerve ligation in the rat, they stud-

ied the vascular effects on the contralateral hind paw. They found evidence for induction of a vasodilator response involvement by inhibition of skin vasoconstrictor activity, as well as antidromically acting sensory nerve fibers. The involvement of the vascular effects on the contralateral side in the above-mentioned model were explained in more detail in their following publication (Kurvers et al. 1996b). They concluded that partial injury of the rat sciatic nerve causes an ipsilateral increase in skin blood flow at an early stage, which is followed by a decrease at a later stage. At both stages, antidromically acting sensory and orthodromically acting nonsensory (sympathetic) nerve fibers are involved in the vasodilator response (Kurvers et al. 1996c). At a later stage, however, neurogenic vasodilator mechanisms are overruled by a nonneurogenic vasoconstrictor mechanism. The latter may consist of supersensitivity of skin microvessels to catecholamines consequent to reduced neurogenic disposition of catecholamines. Kurvers et al.(1997a) studied the spinal component to skin blood flow abnormalities in CRPS patients. They tried to determine whether the mechanism of CRPS is a neuropathic pain syndrome characterized by skin blood flow abnormalities associated with sympathetic vasoconstrictor and antidromic vasodilator mechanisms, which are solely of peripheral origin. Or is there an additional spinal component that acts exclusively through neural pathways or also involves humoral pathways. They concluded in their study that there are indications that there is a spinal component to microcirculatory abnormalities at stage I of CRPS that most likely acts through neural (antidromic vasodilator) mechanisms and that may be initiated by traumatic excitation of a peripheral nerve on the clinically affected side.

9.4 Effect of ketanserin and carnitine on the skin temperature, plethysmogram and pulse oximetry with 40% oxygen compared with 21% oxygen

As Goris et al. (1987) pointed out, in case of a CRPS extremity we have a serious disturbance in the oxygen extraction which is the consequence of shunting of the arterial blood through the affected extremity and which leads to a lowering of the available oxygen in the cells. This shunting will not be expressed in the SpO_2 and will be normal in the untreated CRPS extremity. But when we relieve the vasoconstriction with ketanserin, the oxygen will become available for the cells and we then see a diminishing of the SpO_2 from an average of 100%–98% down to 96%–95%. To investigate that this phenomenon is not a ventilatory effect, we performed a test with 40% oxygen instead of the normal environmental concentration of 21% in the same CRPS patient (see Fig. 26).

We observed that with 40% oxygen the decrease in saturation after ketanserin was smaller in the same patient than with 21% oxygen while the plethymographic changes were in the same magnitude; therefore, ketanserin is relieving the peripheral oxygen shunting resulting in an increase in oxygen extraction. This test points in the direction that the transport of oxygen from the lungs to the peripheral area is not affected by ketanserin and the recorded decrease in SpO2 is the result of an increase in oxygen extraction in the affected treated peripheral area. This experiment confirms the statement of Goris et al. (1987) that there is a diminished oxygen extraction in the area of CRPS, but also gives evidence that ketanserin is relieving this phenomenon.

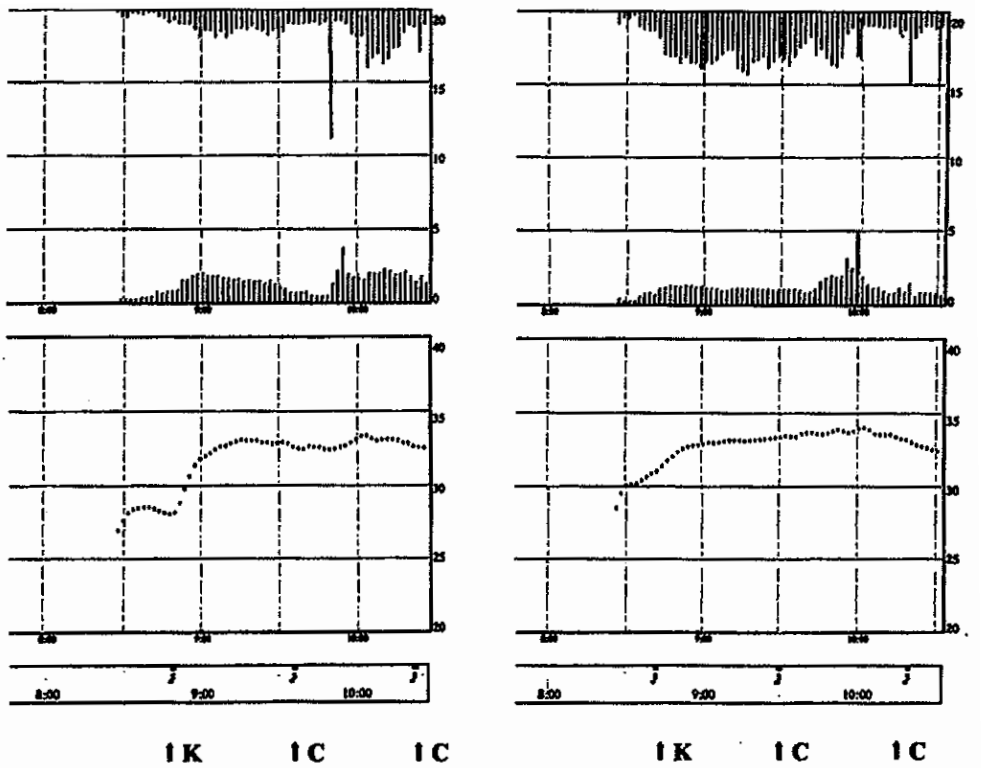

Fig. 26. Trend recording of skin temperature, plethysmography and pulse oximetry (as in Fig. 23) with 21% oxygen (right) and with 40% oxygen (left) on the affected CRPS side.

9.5 Discussion

In the studies on CRPS pathophysiology, dilatatory vessel wall changes accompanied by skin temperature changes and changes in oxygen delivery in the tissues of the affected areas play an important role. Rosén et al. (1988) published the results of LASER Doppler fluxmetry in CRPS. They concluded that an impaired vasomotor reflex response in CRPS is responsible for several clinical features of the syndrome. Slagsvold et al.(1991) studied the relationship between the capillary vessel wall structure of the skin and oxygen reappearance time and oxygen recovery index. They concluded that the influence of structural capillary changes on the transcutaneous pO_2 levels probably increases with the extent of morphological deterioration.

As reported previously (Moesker et al. 1985, 1986, 1998 a, b, c; Moesker 1991, 1995), ketanserin is a very effective drug to relieve the vasoconstriction in CRPS. However, there is no evidence that restoring the circulation with ketanserin relieves the hyperpathic or allodynic pain. Our first observation with carnitine was that, without first restoring the circulation in the ischemic area, no effect was seen on the plethysmogram or in the skin temperature recording. Only after ketanserin infusion, which is known to

result in vasodilatation and an increase in skin temperature, we observed a beneficial effect after the carnitine injection on skin temperature and plethysmogram.

Our results are in line with the theory of Hülsmann (1997) (see Chapter 8). He observed in isolated perfused rat hearts restoration by maintenance of flow and metabolism, after an ischemic period, during suppletion of carnitine.

9.6 Conclusions

Multi-factorial mechanisms are operative after ischemia followed by reperfusion, and these are also present in CRPS. As essential factors we can recognize release of ATP and adenosine production and their effects on purino-receptors, catecholamine release and sensitivity, and neuronal degenerative effects. Ketanserin, a 5-HT_{2A} receptor blocker, relieves the vasoconstrictive effect of serotonin. In patients with CRPS we have proven that this pharmacological approach is effective. In this way it is possible for another drug, carnitine, to reach the ischemic area. This causes extra vasodilatation, probably by the release of ATP to the extracellular space and an increase of the production of adenosine. From the clinical viewpoint, edema, stiffness and ischemic pain are relieved; however, the specific hyperpathic or allodynic pain remains. But, as reported previously (Moesker 1998a, 1998b), continuation of oral treatment with ketanserin and carnitine for several months, can relieve the hyperpathic and allodynic pain in patients with CRPS.

Chapter 10 CARNITINE PLASMA LEVELS AND THE FATE OF INJECTED CARNITINE IN 8 HEALTHY FEMALE VOLUNTEERS

10.1 Introduction

The concentration of total carnitine (TC) in plasma of female adults ranges from 20 to 60 μM. Free carnitine (FC) represents about 75% of the total amount. The remaining esterified fraction, acyl-L-carnitine (AC), contains mainly acetylcarnitine (C2-C). Approximately 75% of the human carnitine requirement is derived from food, meat is by far the richest source, while milk, rice and bread contain much less. The remaining 25% is endogenously synthesized. In humans, the total carnitine concentration ranges from 0.001 μmol/ml in cerebrospinal fluid to about 4 μmol/g in skeletal muscle. Due to the large volume and high carnitine concentration of skeletal muscle, 95% of the total body carnitine (approximately 100 mmol in an adult of 70 kg) is stored in muscle (Rudman et al. 1977, Scholte and de Jong 1987, Di Donato et al. 1984).

Scholte and de Jong (1987) stated that the simplest indication of the carnitine status is plasma FC and TC. When the percentage of FC as a fraction of TC (% FC) drops below 63%, the possibility of secondary carnitine deficiency must be considered. When the level of FC is higher than 25 nmol/ml the carnitine status should be regarded as normal. When the level drops below 25 nmol/ml the risk of functional carnitine deficiency increases (Scholte and de Jong 1987).

10.2 Assay of free carnitine and acylcarnitines

Acylcarnitines and free carnitine were measured by Vreken and coworkers (Vreken et al. 1999) in the Academic Medical Center, University of Amsterdam, Dept. of Clinical Chemistry and Emma Children's Hospital, Laboratory for Genetic Metabolic Diseases Amsterdam, the Netherlands. To 50 μL plasma or serum, 50 μL standard 1 (23.5 μmol/L$[^2H]_3$-L-carnitine in H_2O) and 50 μL standard 2 (10 μmol/L $[^2H]_3$-C_2 carnitine, 2 μmol/L $[^2H]_3$-C_8 carnitine and 2 μmol/L $[^2H]_3$-C_{16} carnitine in acetonitrile) were added. Samples were mixed and subsequently deproteinized with 500 μL acetonitrile and centrifuged. The resulting supernatant was dried under nitrogen at 45°C, and derivatized in 100 μL butanolic-HCl for 15 min at 60°C. Samples were dried under nitrogen at 45°C and redissolved in 300 μL acetonitrile. Prior to injection, 70 μL of acetonitrile containing the acylcarnitines were mixed with 30 μL H_2O. Carnitine and its esters were measured using scanning for precursor ions of mass 85 from 200–550 Da during 2 minutes on a Micromass Quatro II triple-quadrupole mass spectrometer, using a Gilson 231XL autosampler and a Hewlett Packard HP-1100 HPLC pump, essentially as described previously (Rashed et al. 1995, 1997). Calibration curves were obtained for free carnitine in the range of 5-100 μmol/L, 2–40 μmol/L for acetylcarnitine, and from 0.25–6 μmol/L for all other available acylcarnitines by adding deuterated standards to a normal plasma pool. All calibration curves were linear ($r>0.99$, data not shown).

For unsaturated and hydroxylated acylcarnitines a response identical to their saturated counterparts was assumed.

Reproducibility was tested by analysing a spiked plasma pool on different days (n=15) and found acceptable with a day-to-day-coefficient of variation of 8.6% for free carnitine and 6–15% for the different acylcarnitines (data not shown).

The plasma level determinations of Table XVIII were performed in the Department of Pharmacokinetics and Metabolism (Dr. S. Pace) of Sigma Tau Pomezia, Italy, by high-performance liquid chromatography tandem mass spectrometry (Tallarico et al. 1998).

10.3 Control plasma levels from the literature

Table XV summarises the average plasma levels of studies with a relatively high number of normal subjects. Every study showed that FC and TC is higher in males than in females. There was, however, a large variation between the different values. The carnitine levels were the highest in the study of Kuratsune et al.(1994), they reported that AC was statistically significant lower in men. Majeed et al. (1995) found much lower AC levels than the others, and consequently the highest % FC. This was not confirmed in the other studies. It is likely that differences in the methodology give rise to these discrepancies. Since AC is calculated from FC and TC, small errors in the latter values, give rise to large errors in the former value. Another point may be that Kuratsune et al. (1994) used a relatively new spectroscopical method. The other groups used the enzymatic radiochemicalassay. Finally, Scholte et al. (1996), showed that FC levels decreased with time. In 1978, FC in males was 43.4±10 nmol/ml (n=44) and in women 37.0±7.2 nmol/ml (n=46). In this respect it is of interest that the control values reported by Plioplys and Plioplys (1995), are in fact those of a study published 12 years earlier (Rebouche and Engel, 1983).

Table XV. Plasma carnitine levels in controls given in nmol/ml.

First author, year	Gender, n	TC	FC	AC	% FC
Kuratsune, 1994	M, 177	69.5	56.1±10.7	13.4±4.6	81
	F, 131	59.1	43.6±10.0	15.5±4.4	74
Majeed, 1995	M+F, 80	45.9±9.3	39.1±8.2	6.7±2.2	87
Plioplys, 1995*	M, 40	59.3±11.9	46.8±10.0	12.5	79
	F, 45	51.5±11.6	40.1±9.5	1.4	78
Scholte, 1996**	M, 84	49.8±10.0	37.4±8.0	12.5±3.8	75.0±5.5
	F, 57	42.8±8.2	31.1±6.1	11.7±3.7	72.9±6.5

n=number of controls. % FC is FC/TC.100%.
The means are given with SD. Data without SD were not present in the article and calculated.
* Control values are from Rebouche and Engel (1983).
** AC, TC and % FC were obtained as personal communication.

10.4 Control plasma carnitine levels in healthy women

Table XVI lists the control values of 8 healthy women, and Figures 27 and 28 show the levels of TC and FC in relation to age. Random blood samples were taken at normal daily hours, without fasting. It is remarkable that the lowest values were found in the youngest women (aged 19, 27 and 32 years) and the highest value in a healthy 70-year-old volunteer. The mean values for TC, FC and AC were lower than those reported by Scholte et al. (1996). Up to now, although different levels of carnitine and its metabolites have been reported, no correlation between these levels and age has been made. Therefore, we performed statistical regression analysis to establish the relationship between the levels found in our study and age.

Table XVI. Control values (nmol/ml) of 8 healthy women.

	Age (y)	TC	FC	AC	C2-C	C3-C	% FC
F	19	34.4	26.8	7.6	5.5	0.40	78
A	27	31.5	21.2	10.4	7.3	0.15	67
C	32	19.5	16.2	3.3	2.0	0.20	83
G	42	36.6	29.4	7.2	5.0	0.25	80
B	44	43.9	36.9	7.0	4.8	0.50	84
E	48	36.0	30.3	5.6	3.6	0.40	84
D	56	39.9	28.0	11.9	8.8	0.25	70
S	70	57.0	46.0	11.0	7.4	0.50	80
mean	42.2	37.4	29.4	8.0	5.6	0.33	78.2
SD	16.3	10.7	9.1	2.9	2.2	0.14	6.4

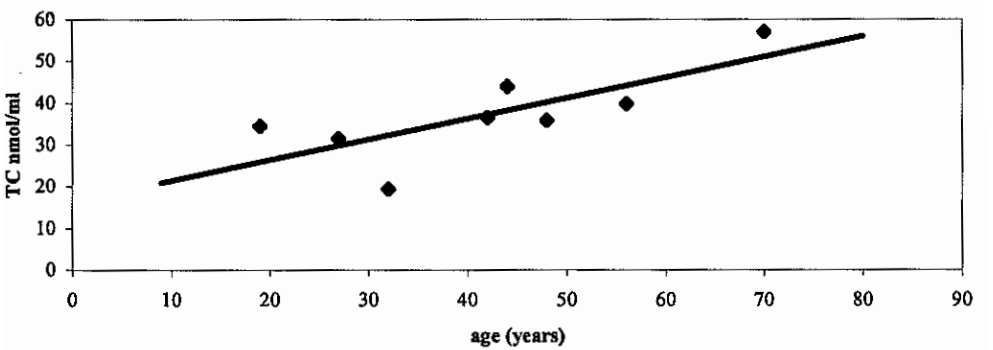

Fig. 27. Relationship between the level of total-L-carnitine and age in 8 healthy women.

10.5 (Acyl) carnitine levels in relationship to age

Our analysis showed the relationship to be: TC= [(0.49 ± 0.18).age] + (16.5 ± 7.6), with a correlation coefficient r of 0.75 (Fig.27). Thus the TC level is increasing 0.49 ± 0.18 nmol/ml with each year of age. There was a positive significant correlation ($p<0.05$) in the relationship between age and TC level. By extrapolation, the estimated TC level at the age of 0 is 16.5 ± 7.6 nmol/ml. Scholte et al. (1996) published levels of 1994 in cord blood, 21.4 ± 4.5 nmol/ml, with a range of 13.3 – 28.8 nmol/ml (n=20).

The relationship for FC levels was: FC = [(0.42 ± 0.15). age] + (11.7 ± 6.6), with a correlation coefficient r of 0.75. There was a significant positive correlation ($p<0.05$) between FC and age (Fig. 28).

The values for AC, C2-C, C3-C and FC as a percentage of TC did not have a significant correlation with age. These values are summarized in Table XVII.

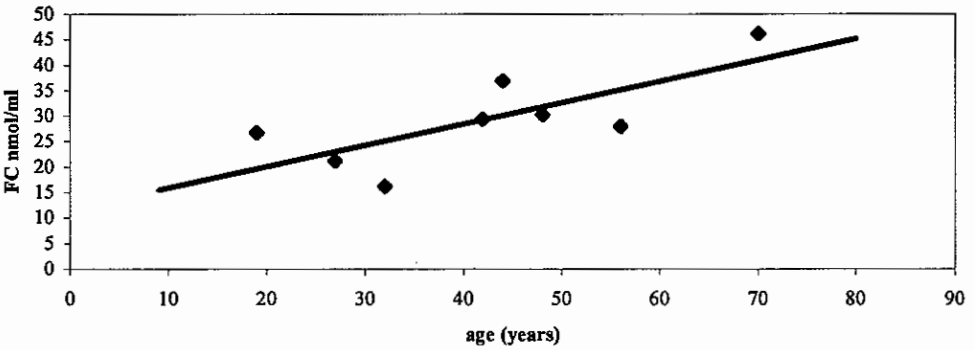

Fig. 28. Relationship between the level of free L-carnitine and age in 8 healthy women.

Table XVII. Sum of values found by statistical analysis in 8 healthy women. X = increase per year of age ± SD, C = extrapolated level at 0 year.

	TC nmol/ml, SD	FC nmol/ml, SD	AC nmol/ml, SD	C2-C nmol/ml, SD	C3-C nmol/ml, SD	% FC, SD
X	0.49 ± 0.18	0.42 ± 0.15	0.08 ± 0.07	0.05 ± 0.05	0.004 ± 0.003	0.057 ± 0.16
C	16.5 ± 7.6	11.7 ± 6.6	4.8 ± 2.85	3.4 ± 2.2	0.18 ± 0.13	75.9 ± 6.9
p	< 0.05	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05

In conclusion, in the case of 8 healthy women (age range 19–70 years) there was a positive significant correlation between the level of FC (range 19.5–57.0 nmol/ml) and age. The levels of AC (range 3.3–11.9 nmol/ml), C2-C (range 2.0–8.8 nmol/ml) and of C3-C (range 0.15–0.50 nmol/ml) had no significant relation with age.

10.6 Effect of ketanserin on (acyl) carnitine levels

In our clinical (experimental) work treating CRPS patients we combined ketanserin with carnitine, with intravenous ketanserin always preceding carnitine. In this way we evaluated the possible effect of ketanserin on the level of carnitine and its metabolites (Table XVIII). There was no effect on the overall levels. To get information about the relationship between the levels and age, we calculated the percentage of change brought about by ketanserin (see Figures 29, 30 and Table XVIII).

Table XVIII. Levels of TC, FC, AC, C2-C, C3-C (nmol/ml) and % FC in 8 healthy women 45 minutes after 10 mg ketanserin i.v.

	Age (y)	TC	FC	AC	C2-C	C3-C	% FC
F	19	37.8	31.3	6.5	4.4	0.45	83
A	27	33.4	23.0	10.3	7.8	0.20	69
C	32	20.3	17.2	3.1	1.8	0.20	85
G	42	37.7	30.0	6.9	4.9	0.30	82
B	44	42.7	36.4	6.2	4.3	0.35	85
E	48	35.0	29.9	5.2	3.1	0.45	85
D	56	38.3	28.9	9.4	7.1	0.20	76
S	70	55.9	45.8	1.01	6.3	0.50	74
mean	42.2	37.6	30.3	7.2	5.0	0.33	79.9
SD	16.4	9.9	8.5	2.5	2.0	0.12	6.1

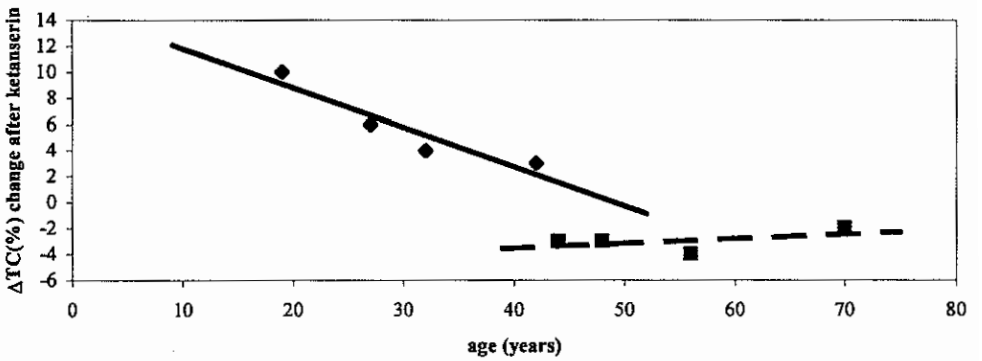

Fig. 29. Percentage change of TC level in plasma related to age in 8 healthy women 45 minutes after 10 mg i.v. ketanserin. TC before ketanserin was taken as 100% [TC in %. Before ketanserin 100%, ΔTC(%)]. The regression lines were calculated for the age group of 19–42 years and for the age group of 44–70 years.

There were important differences with age. After ketanserin the TC levels were increased in the women aged 19 to 42 years, decreasing with age. The levels in those aged over 44 years were slightly decreased. The results showed increased levels in those aged 19 to 42 years and slightly decreased levels in those over 44 years of age. In the age groups

19 to 42 years we found the formula: Δ TC(%) change = [(-0.30 ± 0.08). age] + (14.8 ± 1.3), with a correlation coefficient r of 0.937 ($p<0.05$). For the age group 44 to 70 years we found the formula: ΔTC(%) change = [(0.04 ± 0.04). age] + (-4.9 ± 0.9), with a correlation coefficient r of 0.498 ($p>0.05$).

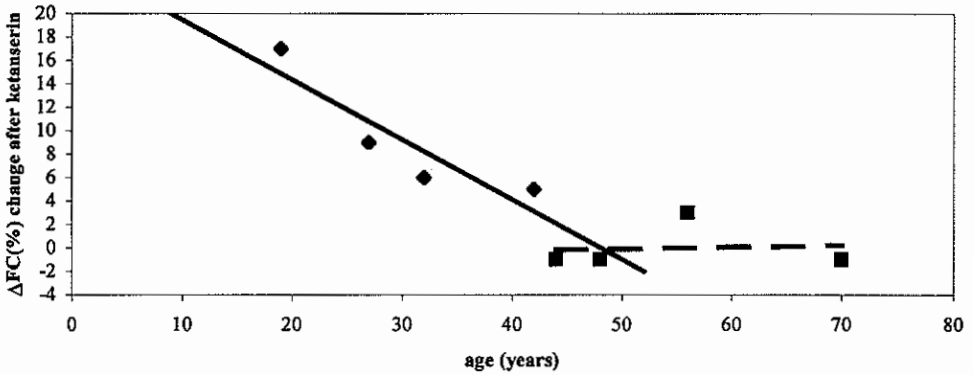

Fig. 30. Percentage change of FC level [(FC in %. Before ketanserin 100%, in short ΔFC(%)] in plasma related to age in 8 healthy women 45 minutes after 10 mg i.v. ketanserin. FC before ketanserin was taken as 100%. Regression lines were calculated as in Fig.29.

We obtained similar results for the FC levels (Fig. 30). For the change of FC level in the 4 women aged less than 44 years we found the formula: ΔFC (%)change = [(-0.51 ± 0.17). age] + (24.57 ± 2.8), with a correlation coefficient r of 0.904. Statistical analyses of the older age levels and for the values of AC, C2-C, C3-C and the change of %FC from TC, revealed no statistical relevant correlations. The results are summarised in Table XIX.

Table XIX. Summary of the statistical analyses of the changes in levels 45 minutes after 10 mg i.v. ketanserin. X = change per year ± SD, C = extrapolated change at 0 year ± SD.

	ΔTC(%) 19–42	ΔTC(%) 44–70	ΔFC(%) 19–42	ΔFC(%) 44–70
X	-0.302 ±0.08	0.035±0.04	-0.511±0.17	-0.015±0.12
C	14.8±1.3	-4.9±0.92	4.6±2.8	-0.8±2.4
p	<0.05	>0.05	<0.05	>0.05

It is interesting that up to 42 years of age ketanserin caused an increase in TC and FC plasma values, whereas above age 44 years these values were slightly decreased. Thus an explanation based on dilution of plasma levels, by vasodilatation alone, can not explain these changes. The change in carnitine plasma levels may be caused by an effect of ketanserin on the active transport of carnitine over the plasma membrane (see Chapter 8, section 8.3). The differences between the age groups of women may be related to the hormonal status.

10.7 Effect of 1 g i.v. L-carnitine on (acyl) carnitine levels after 1 hour

Because we treated our CRPS patients with a loading dose of 1 g carnitine, we evaluated the effect of 1 g carnitine i.v. in the 8 women (Table XX). The same procedure was followed as in the treatment of CRPS patients: stabilisation, 10 mg i.v. ketanserin , after 1 hour 1 g i.v. carnitine, one hour later blood samples were taken. The samples were taken in the afternoon on a normal day without fasting.

Table XX. Levels of TC, FC, AC, C2-C ,C3-C (nmol/ml) and %FC in plasma, in 8 healthy female volunteers, 1 hour after 1 gram i.v. carnitine.

	Age (y)	TC	FC	AC	C2-C	C3-C	% FC
F	19	237.6	228.7	8.9	6.25	0.70	96
A	27	261.4	251.9	9.4	7.25	0.45	96
C	32	170.5	165.7	4.8	3.1	0.45	97
G	42	324.7	315.4	9.6	6.6	0.35	97
B	44	246.4	237.8	8.6	5.9	0.60	97
E	48	323.4	316.1	7.4	5.1	0.60	98
D	56	284.2	273.8	10.5	7.65	0.25	98
S	70	357.5	343.8	13.7	10.45	0.50	96
mean	42.2	275.7	266.6	9.1	6.5	0.49	96.9
SD	16.3	59.8	57.9	2.5	2.1	0.14	0.8

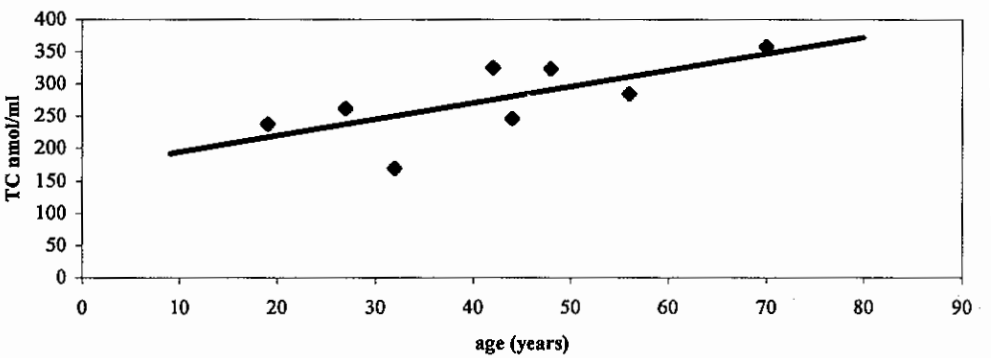

Fig. 31. Relationship between age and levels of TC in 8 women, 1 hour after 1 gram i.v. carnitine.

After estimations of the levels after 1 g i.v. carnitine, we evaluated the relationship between these levels and age by regression analyses.

The formula for the relationship of the TC level 1 hour after 1 gram i.v. carnitine was: TC = [(2.6 ± 1.1).age] + (167.8 ± 46.3), with a correlation coefficient r of 0.70 (Fig. 31). Thus, by extrapolation, at age 0 for TC 1 hour after 1 gram i.v. carnitine, the level is 168 ± 46 nmol/ml. The TC level is increasing by 2.6 ± 1.1 nmol/ml per year of age; this relationship was significant ($p<0.05$).

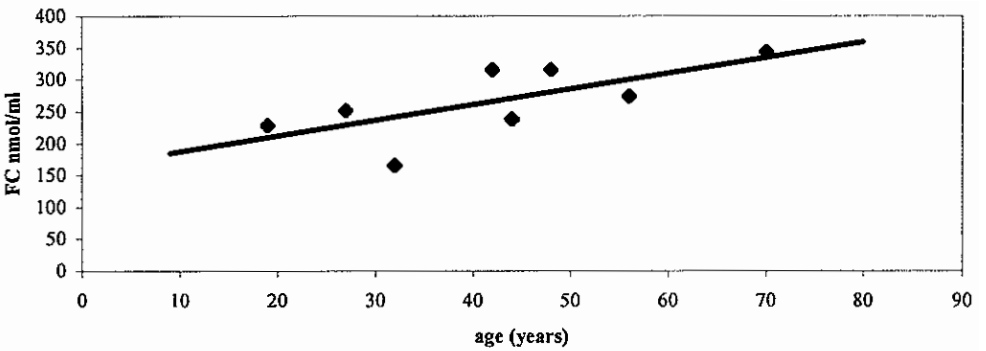

Fig. 32. Relationship between age and levels of FC, in 8 women, 1 hour after 1 gram i.v. carnitine.

In the regression analysis between the levels of FC in plasma in the 8 women we found the formula: FC = [(2.5 ± 1.0). age] + (162.8 ± 45.1), with a correlation coefficient r of 0.694 (Fig.32). The relationship between FC level and age was significant ($p<0.05$).

The relationship between the levels of AC, C2-C, C3-C and FC as a fraction of TC showed no significant correlation with age (Table XXI).

Table XXI. Summary of the statistical values found in 8 healthy women 1 hour after 1 gram i.v. carnitine. X = level change per year of age ± SD. C = extrapolated level at 0 year ± SD.

	TC	FC	AC	C2-C	C3-C	% FC
X	2.6±1.1	2.5±1	0.1±0.05	0.1±0.04	-0.003±0.003	0.02±0.02
C	167.8±46.3	162.8±45.1	5.1±2.2	3.3±1.8	0.62±0.15	96.2±0.9
p	<0.05	<0.05	>0.05	>0.05	>0.05	>0.05

It is remarkable that 1 hour after 1 g i.v. carnitine the significant age-related correlation between TC and FC is restored, despite the fact that the mean level of TC and FC is more than 6 times higher than the mean starting levels.

10.8 Pharmacodynamic profile of 1 g L-carnitine i.v. during 1 hour

To gain information about the level changes in the first hour after the injection of 1 g i.v. carnitine, blood samples were taken 2, 5, 10, 30 and 60 minutes after the injection. The pharmocodynamic profiles are given in Figures 33 to 37.

Figures 33 and 34 show that after 1 g i.v. carnitine the highest levels of TC (Fig.33) and FC (Fig.34) are reached in 4 persons after 2 minutes, in 3 persons after 5 minutes and in 1 person after 10 minutes. Highest levels are reached in 7 women between 2 and 5 minutes; only in one woman the highest level was reached after 10 minutes. Apparently, the kinetics of the processes involved in the age-related carnitine homeostasis, are different. At 30 and 60 minutes the plasma levels of TC and FC decrease with average rates of 2.57 (±0.89) and 2.59 (±0.90) nmol/(ml.min). The mean value for TC increased from 37.6 (±9.9) to 278.0 (±58.8), which is a mean increase of 710%. For FC we

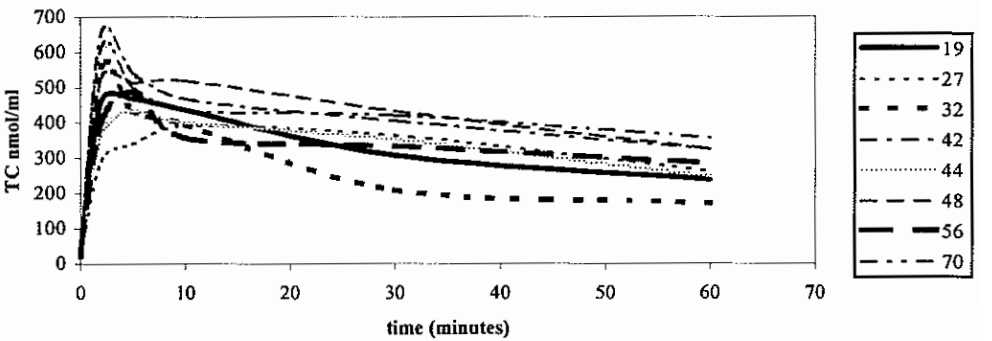

Fig. 33. Levels of TC in 8 women, 2, 5, 10, 30, 60 minutes after 1 gram i.v. carnitine. The legend on the right indicates which line corresponds with the age.

calculated an increase of 30.3 (±8.5) to 266.6 (±58.0), which is a mean increase of 880%.

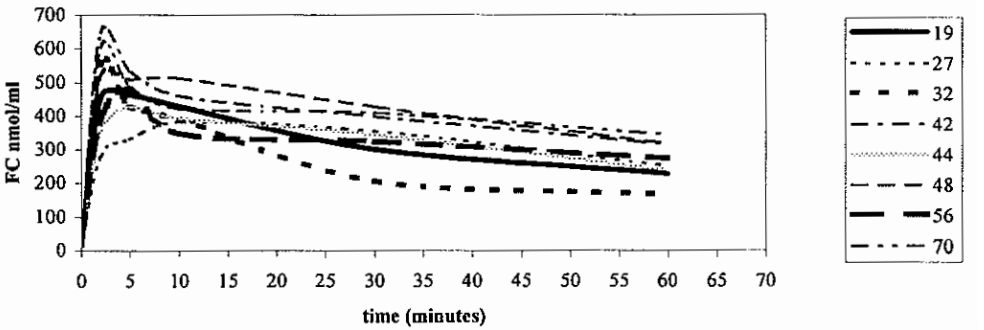

Fig. 34. Relationship of free L-carnitine (FC) levels in 8 women 2, 5, 10, 30 and 60 minutes after 1 gram i.v. carnitine.

Figure 35 shows the levels of AC at 2, 5, 10, 30, and 60 minutes after 1 gram i.v. carnitine.

In 7 out of 8 women we found an increase in the level of AC at 60 minutes after 1 gram i.v. carnitine. The mean value increased from 7.2 ± 2.4 nmol/ml to 9.1 ± 2.4 nmol/ml after 60 minutes, which is a mean increase of 26%. After 2 minutes the level of AC decreased in 3 women, increased in 4, and remained unchanged in 1 woman. After 10 minutes, 1 woman still had a decreased level which persisted for 60 minutes.

As expected C2-C showed a similar fate as AC (Fig.36). The mean C2-C level increased from 4.9 ± 1.9 nmol/ml to 6.5 ± 2.0 nmol/ml, which is a mean increase of 30%.

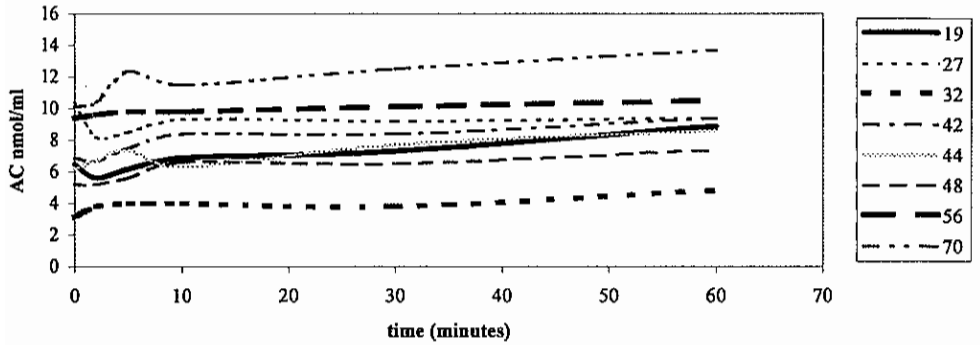

Fig. 35. Levels in plasma of acyl-L-carnitine (AC) in 8 women at 2, 5, 10, 30, and 60 minutes after 1 gram i.v. carnitine.

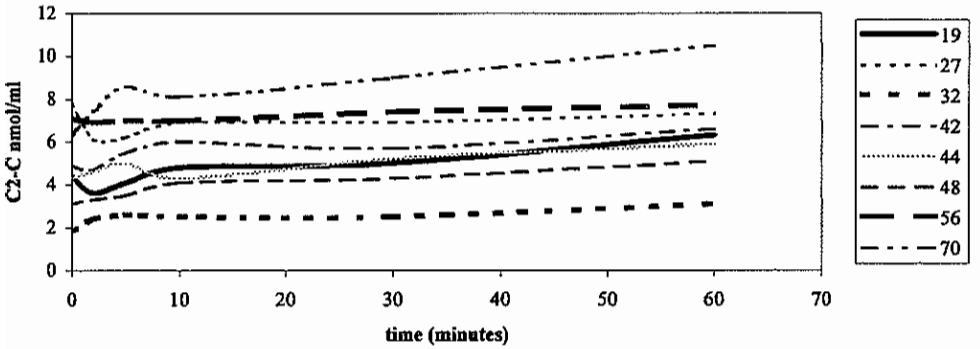

Fig. 36. Levels of acetyl-L-carnitine (C2-C) in 8 women at 2, 5, 10, 30, and 60 minutes after 1 gram i.v. carnitine.

Figure 37 shows the levels of C3-C at 2, 5, 10, 30, and 60 minutes after 1 g i.v. carnitine. All women, except 1, showed an increased level after 60 minutes. The mean increase was from 0.3 ± 0.1 to 0.5 ± 0.1 which is a mean increase of 48%. The average AC, C2-C and C3-C levels were still increasing after 1 h. So the esterification plus export is much slower than the import of carnitine out of the plasma into the cells.

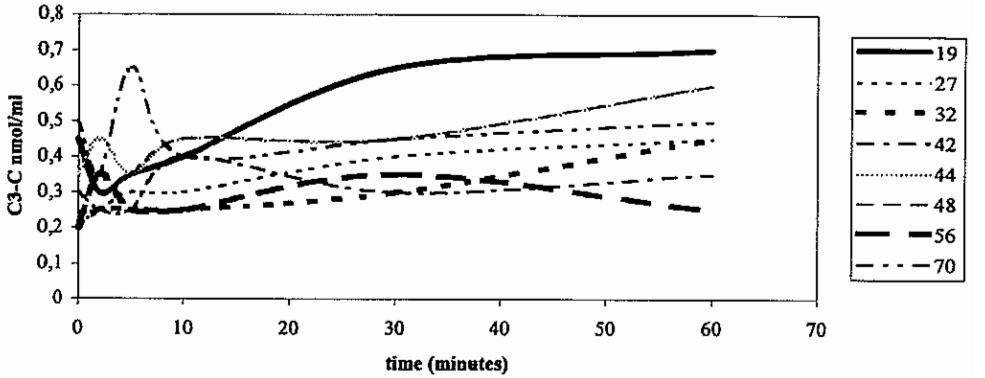

Fig. 37. Levels of propionyl-L-carnitine (C3-C) in 8 women at 2, 5, 10, 30 and 60 minutes after 1 gram i.v. carnitine.

In conclusion, we observed in 8 healthy women that:

1. The levels of plasma TC and FC increased with age.
2. Intravenous ketanserin in the younger group (19–42 years), caused an increase of plasma TC and FC level. This effect decreased with age. In older women (44–70 years) ketanserin caused a slight decrease in the levels.
3. Intravenous L-carnitine in a dosage of 1 g, caused after 1 h increased levels of TC (to 710%) and FC (to 880%) and smaller increases of AC (to 126%), C2-C (to 130%) and C3-C (to 148%).
4. The carnitine homeostasis had the ability to restore the age relationship of the TC and FC levels within 60 minutes after the carnitine injection.

Chapter 11 CARNITINE PLASMA LEVELS AND THE FATE OF INJECTED CARNITINE IN CRPS PATIENTS

11.1 (Acyl)carnitine plasma levels in CRPS patients

Our carnitine level estimations and those of the group of patients reported by Van Oudheusden for younger girls (personal communication) enabled us to determine the relationships between age and carnitine and its acylcarnitines in female CRPS patients. All blood samples were taken before any drug treatment was started. Figure 38 shows the relationship between TC plasma level and age in 45 CRPS patients.

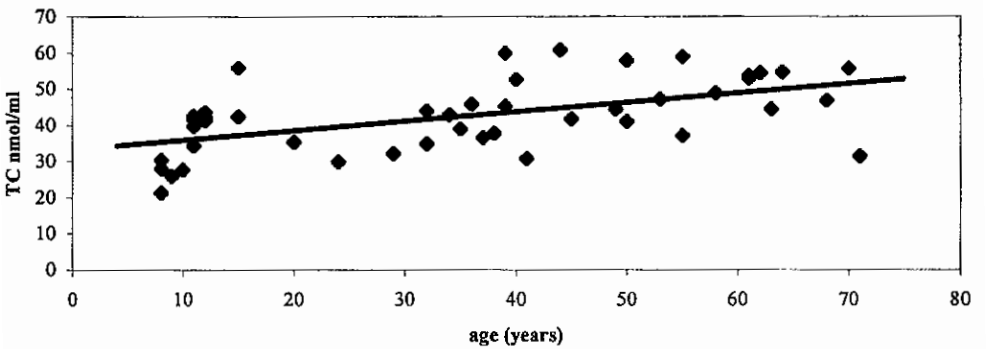

Fig. 38. Relationship between TC level and age. The diamonds are TC values of different patients around the regression line, n=45.

The relationship between TC level and age was: TC = [(0.26 ± 0.06). age] + (33.2 ± 8.5), with a correlation coefficient r of 0.53; in case of n = 45, there was a positive significant correlation ($p<0.05$) in this relationship. Thus, we can state that 28% of the increase in the total L-carnitine level with increasing age in this group of CRPS patients is dependent on the factor age. Figure 39 shows the relationship between free L-carnitine level and age in 51 CRPS patients.

The relationship between FC level and age in CRPS patients was: FC = [(0.20 ± 0.05). age] + (26.0 ± 7.1), with a correlation coefficient r of 0.51. In case of n=51, there is a significant positive correlation ($p<0.05$) between FC levels and age. Thus, 26% of the increasing level of free L-carnitine is correlated with increasing age.

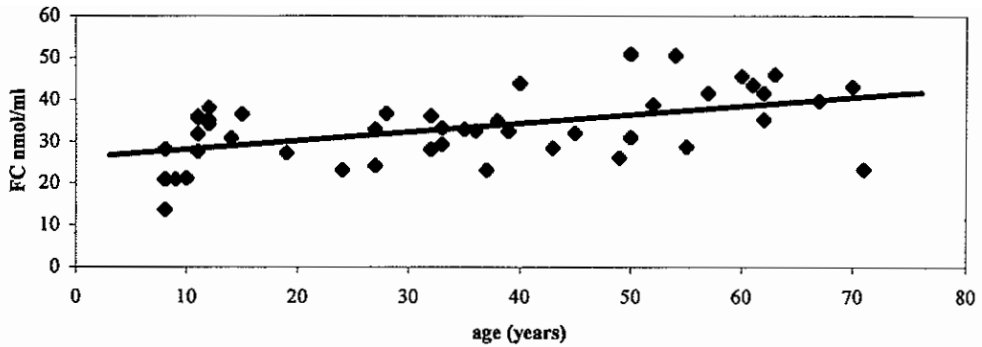

Fig. 39. Relationship between FC level and age (n=51).

Figure 40 shows the relationship between the acyl-L-carnitine plasma level and age in 45 CRPS patients. The relationship for AC levels was: AC = [(0.07 ± 0.02). age] + (5.92 ± 2.78), with a correlation coefficient r of 0.45. In case of n=45, there was a positive significant correlation between AC level and age, that 20% of the increasing level of acyl-L-carnitine is correlated with increasing age.The values of C2-C, C3-C and %FC had no significant correlation with age. All the values are summarised in Table XXII.

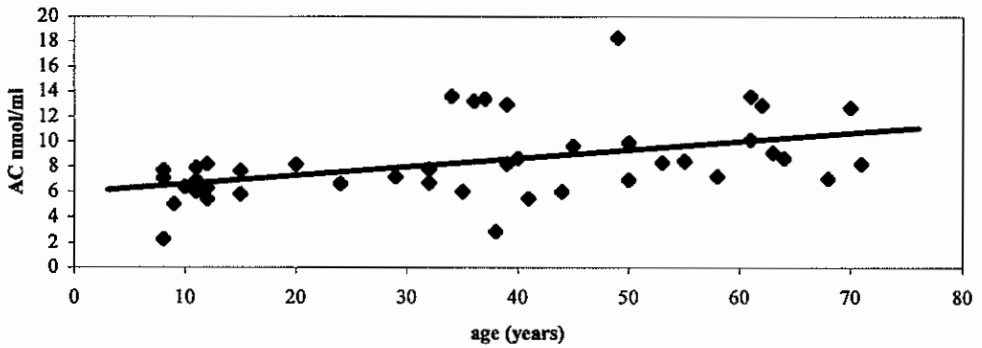

Fig. 40. Relationship between AC level and age in CRPS patients (n=45).

Table XXII. Sum of the values found by statistical analyses in CRPS patients. X = increase per year ± SD, C = extrapolated level at 0 year ± SD.

	TC nmol/ml±SD	FC nmol/ml±SD	AC nmol/ml±SD	C2-C nmol/ml±SD	C3-C nmol/ml±SD	% FC/SD
X	0.26 ± 0.06	0.02 ± 0.05	0.07 ± 0.02	0.03 ± 0.06	0.002 ± 0.002	0.08 ± 0.07
C	33.2 ± 8.52	6.0 ± 7.1	5.9 ± 2.8	4.6 ± 1.7	0.35 ± 0.09	82.0 ± 8.4
p	< 0.05	< 0.05	< 0.05	> 0.05	> 0.05	> 0.05

11.2 Effect of 1 g L-carnitine i.v. on (acyl)carnitine levels after 1 h in CRPS patients

In the group of healthy volunteers (controls) we investigated the pharmacodynamic profile in the first hour after 1 gram i.v. carnitine. Because we specifically evaluated the effect at the end of the first hour in controls, we also evaluated the effect of 1 gram i.v. carnitine in CRPS patients after one hour. Total L-carnitine level in plasma and the relationship with age one hour after 1 gram i.v. carnitine in 32 CRPS patients is shown in Figure 41. The relationship was: TC = [(1.33 ± 0.79). age] + (199.5 ± 66.0), with a correlation coefficient r of 0.29. The X-coefficient was positive, so there was a tendency for the TC level to increase with increasing age ($p<0.05$, n=32).

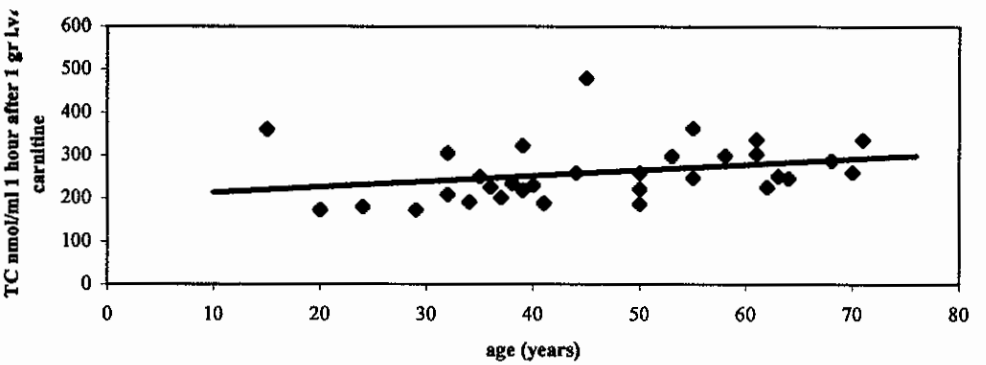

Fig. 41. Relationship between TC level and age one hour after 1 gram i.v. carnitine, (n=32).

Figure 42 shows the relationship between free L-carnitine level in plasma and age one hour after 1 gram i.v. carnitine in 32 CRPS patients. The relationship for FC was: FC = [(1.94 ± 0.77). age] + (150.6 ± 64.5), with a correlation coefficient r of 0.42. In case of n=32 and $p<0.025$ there was a significant correlation between FC level and age, we may state that 18% of the increase in the level of free L-carnitine is related to increasing age. Statistical analyses of the values for AC, C2-C, C3-C, and %FC showed no significant correlation with age. Table XXIII presents a summary of the estimated values.

Table XXIII. Summary of the values of regression analyses of TC, FC, AC, C2-C, C3-C and %FC in CRPS patients, 1 hour after 1 gram i.v. carnitine.

	TC nmol/ml±SD	FC nmol/ml±SD	AC nmol/ml±SD	C2-C nmol/ml±SD	C3-C nmol/ml/±SD	%FC±SD
X	1.33±0.79	1.94±0.77	0.004±0.107	0.025±0.05	0.0005±0.0028	0.001±0.067
C	199.5±66	150.6±64.5	14.3±8.9	8.17±4.38	0.56±0.24	94±5.6
p	<0.05	<0.05	>0.05	>0.05	>0.05	>0.05

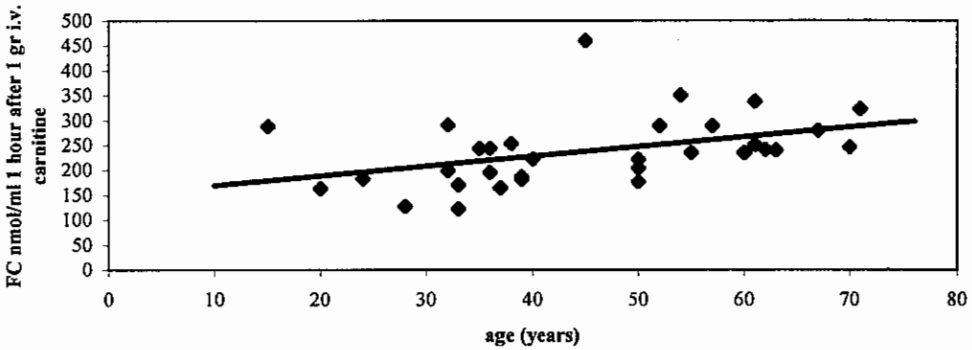

Fig. 42. Relationship between FC level and age one hour after 1 gram i.v. carnitine, (n=32).

11.3 Effect of 9 months L-carnitine medication on (acyl)carnitine levels

To gain more information about the effect of long-term oral carnitine treatment on plasma levels, during CRPS treatment, blood samples were taken after 9 months of L-carnitine medication in combination with ketanserin. As baseline the blood values before start of treatment were used (Table XXIV).

Table XXIV. Data on L-carnitine and its quantitatively most important metabolites at baseline and after 9 months of oral treatment with 3 g L-carnitine daily. * Values before start of treatment (baseline), ** values after 9 months oral treatment with 3 g L-carnitine.

Age (y)	TC*	TC**	FC*	FC**	AC*	AC**	C2-C*	C2-C**	C3-C*	C3-C**	% FC*	% FC**
16	41.0	35.4	30.8	30.8	6.0	4.6	4.2	3.1	0.30	0.3	73	87
21	35.4	48.6	27.3	38.1	8.2	10.6	5.7	7.9	0.45	0.5	79	78
34	42.0	67.9	29.4	58.8	13.6	9.2	9.9	6.5	0.50	0.8	68	87
37	36.6	49.4	23.2	41.8	13.4	7.5	8.9	5.2	0.25	0.5	63	85
49	44.7	74.8	32.1	60.9	12.7	13.9	6.1	10.1	0.50	1.3	72	81
51	57.9	85.1	51.0	74.3	6.9	10.9	5.0	8.3	0.55	0.8	88	87
61	52.9	60.9	39.3	52.6	13.6	8.2	10.2	5.5	0.35	0.6	74	87
62	54.5	59.4	41.6	50.3	12.9	9.1	8.9	6.3	0.50	0.55	77	85
Mean	45.9	60.2	34.3	50.9	10.9	9.2	7.3	6.6	0.43	0.67	74.2	84.6
SD	7.9	14.8	8.4	13.0	3.1	2.5	2.2	2.0	0.10	0.28	7.0	3.2

Concentrations are in nmol/ml.

In our adult population we found increased levels of TC, FC and C3-CC, but decreased levels of AC and C2-C. It is remarkable that Van Oudheusden found increased levels of TC, FC, and AC in 6 children 4-6 months after starting carnitine supplementation (data obtained by personal communication); however these children were not treated with ketanserin.

Analyses with the paired *t*-test (α=0.05, n=8) showed that in our patients after 9 months there was a significant increase in the TC level ($p<0.05$). Similarly after 9 months the *t*-test (α=0.05, n=8) showed a significant increase in the FC level ($p<0.02$). The *t*-test for AC, C2-C and C3-C showed no significant increase. For the increase %FC expressed as a % of TC the *t*-test (α=0.05, n=8) showed a significant increase ($p<0.005$) (Table XXIV).

Table XXV presents data on increased or decreased levels expressed as a percentage of the baseline values of the 8 study patients.

Table XXV. Increase or decrease in plasma levels expressed as a percentage of the baseline values.

	TC	FC	AC	C2-C	C3-C	% FC
Mean (%)	132	151	91	99	157	115
SD	27	35	37	49	53	13

Again, statistical analyses were performed to establish the relationship between L-carnitine and metabolites and age after 9 months oral carnitine treatment. Table XXVI presents an overview of the estimated values. After 9 months oral treatment the relationship between TC, FC and age showed a positive significant correlation.

Table XXVI. Summary of statistical analyses of the values for TC, FC, AC, C2-C, C3-C and % FC after 9 months of oral carnitine treatment.

	TC nmol/ml	FC nmol/ml	AC nmol/ml	C2-C nmol/ml	C3-C nmol/ml	% FC
X	0.575±0.291	0.513±0.254	0.06±0.06	0.04±0.05	0.007±0.007	0.05±0.08
C	36.4±13.3	29.7±11.6	6.76±2.72	4.94±2.20	0.38±0.30	82.5±3.5
p	<0.05	<0.05	>0.05	>0.05	>0.05	>0.05

11.4 Effect of ketanserin on plasma carnitine levels in CRPS patients

In Chapter 10 we evaluated the effect of ketanserin on the plasma carnitine levels in women (controls). Because of the interesting relationship with age and the difference between the plasma levels after ketanserin in the controls under the age of 44 years compared with the effect in women above the age of 44 years, we investigated these components in the plasma levels of carnitine in CRPS patients. Attention was also given to the effect of ketanserin on carnitine plasma levels in a later stage of treatment.

11.4.1 Effect of ketanserin on baseline values of carnitine plasma levels in CRPS patients

Figure 43 shows the percentage change in FC levels [Δ FC(%)] 45 minutes after 10 mg i.v. ketanserin compared to the baseline level in 7 new CRPS patients, who were starting treatment, in relationship to age. These 7 starters (2 males, 5 females) were aged 17 to 60 years (2 males aged 17 and 60 years), all fulfilled the criteria mentioned in Chapter 2 section 2.5 (Table V), thus were diagnosed as CRPS type I. Delay between onset of symptoms and start of treatment was mean 7 (SD 4.5) months. The involved extremities were 6 times a leg and 1 time an arm. As shown in Chapter 10 (Fig. 30) there is a significant correlation between the Δ FC(%) increase after ketanserin in controls, especially in those under 44 years of age. This was not confirmed in the case of CRPS patients. Although there are patients with the expected increase in FC level, we also found patients, in the younger age group, with a decreased FC level after ketanserin. The effect of ketanserin in CRPS patients is, therefore, not always the same as in the controls and there is no significant correlation between these two groups. The severity of the CRPS disease probably has an influence on this phenomenon (see Chapter 13, section 4).

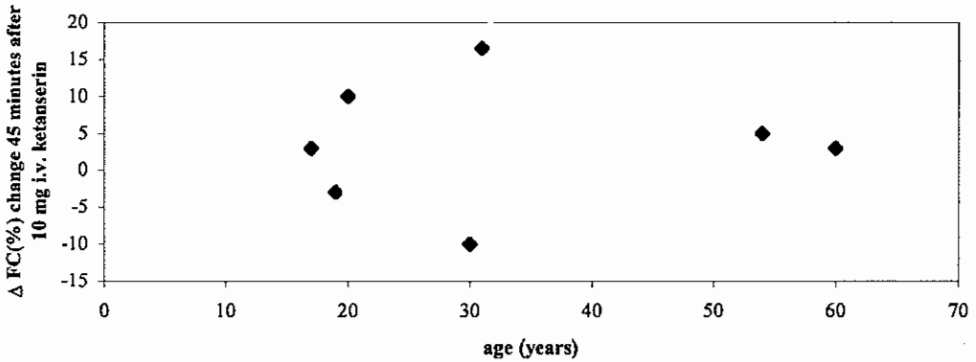

Fig. 43. Effect of 10 mg i.v. ketanserin after 45 minutes as a percentage of baseline FC levels [in %.Before ketanserin 100%, in short Δ FC(%)] in 7 CRPS patients, related to age.

11.4.2 Effect of ketanserin on carnitine plasma levels in a later stage of treatment in CRPS patients

We also investigated the effect of ketanserin after 45 minutes in a later stage, after 6 to 9 months oral treatment (see Fig. 44). These 9 patients (1 male, 8 females) were aged 28 to 63 years (the male patient was aged 38 years). Delay between onset of CRPS symptoms and treatment was mean 11.4 (SD 4.3) months. Extremities involved were 3 times an arm and 6 times a leg. After treatment no significant correlation was detected between Δ FC(%) level change and age.

Fig. 44. Effect of 10 mg i.v. ketanserin after 45 minutes as a percentage of baseline FC levels [Δ FC(%)] in 9 CRPS patients related to age, in a later stage of treatment.

11.5 Conclusions

Comparison the data and statistical analyses from controls with those from CRPS patients allows us to draw the following conclusions. The relationship between plasma levels and age found in controls was similar to that in the CRPS patients. In both groups there was a positive significant correlation between increasing TC and FC levels and age. The total levels of TC, FC, AC, C2-C and C3-C in CRPS patients of all ages were higher than those in controls. In controls and CRPS patients we found that one hour after 1 gram i.v. carnitine the carnitine homeostasis had already re-established the significant correlation between age and level of TC and FC.

Table XXVII presents an overview of the mean values in the different study groups. Because ketanserin had a different effect on TC and FC levels in women aged less than 44 years compared with older women, we averaged the levels for the different groups into those aged < 44 years and those aged 44 years and older. In the CRPS group aged both under and over 44 years of age the average FC levels were higher than those in the controls, the C2-C level was in the same range and the C3-C level was (slightly) higher.

One hour after 1 gram i.v. carnitine there was an age-related change in the plasma FC levels. The youngest group, CRPS patients < 44 years had the lowest FC level, as did the controls in the same age category. The highest levels were found in the highest age category of the control group; the FC levels of the CRPS patients > 44 years, were lower than those of the older control group. The increases in C2-C levels in all the age groups were of the same magnitude. For C3-C we found slightly increased levels 1 hour after 1 g of carnitine, but (as with FC) the highest level was in the oldest age group.

Analyses of the baseline values of the controls with the *t*-test two tailed probability (n=4), showed no significant difference in the FC levels between those aged < 44 years and those aged 44 years and older ($p>0.05$). In CRPS patients (n=16) the *t*-test showed a significant difference in the FC levels ($p<0.05$) between those aged < 44 years and those aged > 44 years. Thus, FC levels in the older age group are higher than those

in the younger age group. One hour after 1 gram i.v. carnitine there was no significant difference in the FC level in the controls. In contrast in the CRPS patients (<44 years and >44 years) 1 hour after 1 gram i.v. carnitine there was a significant difference in the results (*t*-test, n=16); the older group had a significantly higher FC level ($p<0.05$) than the younger group. Statistical analyses of C2-C and C3-C levels in the controls and in CRPS patients, in the different age groups, in baseline values and in values 1 hour after 1 gram i.v. carnitine, showed no significant difference in the *t*-tests of the different age groups.

In the study group undergoing oral carnitine treatment for 9 months, we observed a remarkable phenomenon. As was also found by Van Oudheusden (personal communication) in 6 children after 6 months oral treatment, in our group the FC level had increased after 9 months, whereas the level of C2-C had not increased. Van Oudheusden found an increase in AC. In our study, a possible explanation for this result could be that we made a negative selection of patients, when they still need carnitine treatment after 9 months, they belong to a CRPS group which is difficult to treat. The statement by Maebashi et al. (1976) that carnitine levels in plasma decrease with increasing age may not be true. Our studies show that both the FC level and the level of its esters increase significantly with increasing age.

Table XXVII. Mean values (±SD) of FC, C2-C and C3-C (nmol/ml) in the different study groups. Δ is the difference after 1 gram i.v. L-carnitine.

	n	Age/y	FC	FC + 1 g i.v.	C2-C	C2-C +1 g i.v.	C3-C	C3-C + 1 g i.v.
controls	8	42.3±15.3	28.6±7.2	258±101	5.5±2.1	6.5±2.0	0.33±0.13	0.49±0.14
controls <44 y	4	30.0±8.3	23.4±5.9	240±62	4.7±2.5	5.8±1.6	0.25±0.09	0.49±0.13
				Δ226		Δ0.8		Δ0.24
controls ≥44 y	4	54.4±9.9	35.3±8.1	293±47	5.2±1.8	7.3±2.1	0.41±0.10	0.49±0.14
				Δ268		Δ1.1		Δ0.08
CRPS < 44 y	16	33.2±7.5	31.2±5.5	203±50	5.7±2.1	9.3±4.8	0.42±0.20	0.59±0.26
				Δ164		Δ3.7		Δ0.17
CRPS ≥ 44 y	16	58.0±7.4	38.6±8.4	274±69	6.1±1.4	9.3±3.7	0.47±0.17	0.58±0.20
				Δ225		Δ3.2		Δ0.11

To summarise, we made the following observations in our studies:

1) In female CRPS patients there was a significant positive correlation between plasma TC and FC levels and age, as was previously found in the control group.
2) In young healthy females i.v. ketanserin caused an increase in plasma FC. There was a significant correlation between Δ FC(%) level change and age after ketanserin, with a decreasing increase up to the age of 44 years. This was not the case in CRPS patients.
3) In CRPS patients, after an overload of 1 gram i.v. carnitine, the carnitine regulating system restored the age-dependent order of TC and FC levels after only 1 hour, as was previously found in controls.
4) In CRPS patients the plasma levels of FC were higher than in age-matched controls. The levels of C2-C and C3-C were the same as in age-matched controls.

5) After 9 months oral therapy in CRPS patients, the FC and C3-C levels were increased, whereas the C2-C level remained unchanged.

Taking into account that intracellularly the levels of FC and its metabolites are 10-100 fold that of the plasma levels, and that this is achieved by active transport of carnitine catalysed by the carnitine importer (see Chapter 8, section 8.3), it is likely that this transport protein plays an essential role in the carnitine household and also in the effects observed.

Chapter 12 CLINICAL EFFECTS OF KETANSERIN AND CARNITINE IN CRPS PATIENTS

12.1 Effects of oral ketanserin combined with oral carnitine (pilot study)

In our patients (n=12) the diagnosis of complex regional pain syndrome (CRPS) was made as before. The symptoms were scored at the initiation of the treatment and again after 3 months of combined oral treatment with ketanserin and carnitine. Patient data are given in Table XXVIII.

Table XXVIII. Data on patients in the pilot study receiving oral ketanserin and carnitine medication.

Patient ID	Male/ Female	Age (y)	Duration of complaints (months)	Skin temperature (°C)	Area
A	F	37	13	28.1	Foot
B	F	38	6	29.1	Foot
C	F	39	3	33.6	Hand
D	F	40	30	31.4	Hand
E	F	24	3	28.5	Foot
F	M	48	108	25.9	Foot
G	M	37	192	23.1	Foot
H	M	42	6	29.2	Knee
I	F	55	6	33.0	Hand
J	F	50	3	31.7	Hand
K	F	36	16	25.3	Hand
L	M	42	9	32.5	Foot
Mean		40.7	33		
SD		7.6	5.8		

All patients in this group had previously undergone surgery on the affected area of the hand, foot or knee. Although this group comprised only 12 patients, the general clinical profile corresponded well with that of CRPS patients reported in other larger studies.

The average delay between onset of symptoms and initiation of treatment was 33 months; this long length of time was mainly due to two patients who had a delay of 108 and 192 months, respectively. When omitting these two patients, the mean delay was 9.5 months. To investigate the phenomenon of a warm or a cold CRPS we used the objective measurement of skin temperature. Taking 32 °C as a normal skin temperature, 7 of our patients had cold and 5 had normal/warm extremities. The symptom of abnormal pain feeling, i.e. hyperpathia and/or allodynia was present in 6 of the 12 patients in our pilot study.

All treatment and monitoring was done as described earlier. First, the patients received a bolus of 10 mg ketanserin i.v. in a continuous saline infusion, followed by

ketanserin administered at a rate of 4 mg per hour. One hour later, a bolus injection of 1 g carnitine was given. After this, oral maintenance therapy was started with ketanserin 20 mg three times daily and carnitine 990 mg three times daily, for 3 months.

To compare the clinical manifestations of the CRPS before and after the infusion treatment followed by three months oral therapy, an inventory of the previously described seven CRPS symptoms was made. With help from the pain consultant nurse, the patient graded the symptoms on a 4-point scale: 0 = absent, 1 = mild, 2 = moderate, 3 = severe.

The grading of symptoms at initiation of therapy and the change in symptomatology after 3 months is shown in Tables XXIX and XXX.

Table XXIX. Grading of seven symptoms of CRPS in 12 patients (A to L) at initiation of therapy (see text for grading scale).

	A	B	C	D	E	F	G	H	I	J	K	L
persistent pain at rest	3	3	3	3	3	3	3	3	3	3	2	2
increasing pain during exercise	3	3	3	3	3	3	3	3	3	3	2	3
impaired mobility	3	3	3	3	2	3	2	3	3	3	0	2
edema	2	2	2	2	1	2	1	2	2	2	1	2
hyperhidrosis	2	2	2	2	1	1	2	2	1	1	0	2
abnormal skin temperature	3	3	2	2	1	1	1	2	2	1	1	2
hyperpathia and/or allodynia	2	3	1	1	1	0	2	0	0	0	0	0

Table XXX. CRPS symptomatology three months after infusion treatment followed by three months oral maintenance therapy with ketanserin and carnitine (see text for grading scale).

	A	B	C	D	E	F	G	H	I	J	K	L
persistent pain at rest	0	0	0	0	0	1	0	0	0	0	0	0
increasing pain during exercise	1	0	0	1	1	2	1	1	1	1	1	1
impaired mobility	1	0	0	1	1	2	1	1	1	1	1	1
edema	0	0	1	0	0	0	0	0	0	0	0	0
hyperhidrosis	0	0	1	0	0	0	0	0	0	0	0	0
abnormal skin temperature	0	0	0	0	0	0	0	0	0	0	0	0
hyperpathia and/or allodynia	0	0	0	0	0	0	0	0	0	0	0	0

After the intravenous treatment with ketanserin in this group of 12 patients the overall symptomatology diminished dramatically. All patients had a normalisation of the peripheral circulation of the affected limb, as previously demonstrated by Moesker (1986, 1991, 1995) and Hanna et al. (1989). The symptom of skin temperature was normalised. Patient B was totally cured of all complaints of CRPS, despite severe CRPS that had persisted for three months with a skin temperature of 29.1°C and seriously debilitating allodynia of the affected foot. Patients A, D, E, G, H, I, J, K and L (9/12) improved considerably, but still suffered from mild pain which increased during exercise and impaired mobility. Patient C was a remarkable case, who made a good recovery, but edema and hyperhidrosis persisted. Patient F, who was a severe case with a delay in treatment

time of 108 months, continued to experience the worst symptomatology with pain at rest, pain during exercise and impaired mobility.

It is probable that the treatment period with ketanserin and carnitine was too short to cure the persisting impaired mobility and pain during exercise. Continuation of the treatment and more studies were required to establish whether total relief can be achieved by oral treatment, or by use of more aggressive therapy with intravenous infusion. Subdivision in the three phases, or the division in a warm or cold CRPS at initiation of therapy, did not influence the outcome of diminishing of symptoms. It was remarkable that in all 6 patients with hyperpathia/allodynia, this symptom disappeared. This phenomenon has not been observed before the introduction of carnitine, in spite of the fact that we have treated many CRPS patients over a period of 18 years.

12.2 Effects of ketanserin combined with carnitine (9 months follow-up study)

In the past we learned from the ketanserin therapy the advantage of the use of an intravenous starting dose, before initiation of oral maintenance therapy. Based on these experiences we also began the carnitine therapy with an intravenous starting dose. Because carnitine has no known acute circulatory effects, we started with ketanserin to relieve the vasoconstriction. Vasodilatation is not only necessary to treat the pathophysiology of CRPS, but our results indicated that it created an optimal condition for carnitine to reach the affected area. In this way we intensified the treatment. After starting intravenous ketanserin at a dose of 10 mg (once or twice), the patients received one hour later a first intravenous dose of 1 g carnitine, and after another hour a second dose. Thereafter, oral maintenance therapy was started with daily dosages of ketanserin 20–20–40 mg, and carnitine 3 times 1 g per day. When there was an insufficient effect after two weeks, we intensified the treatment by repeating the intravenous treatment in polyclinic sessions once every two weeks.

Patient data: follow-up study group

This follow-up study group included only those patients suffering from CRPS type I. Patients suffering from CRPS type II, or with CRPS affecting more than one extremity, or those with complaints described as "shoulder-hand syndrome" were excluded. All patients gave informed consent to be treated with ketanserin and carnitine, because neither of these drugs are recommended for the indication of CRPS type I. Patient data are given in Table XXXI.

Table XXXI. Data on the follow-up study group receiving ketanserin/carnitine treatment.

Number of patients	48
Male/Female	6/42
Age (y) -mean ± SD	44.8 ± 13.8
-range	8–71
Duration of complaints (months) - mean ±SD	15.2 ± 14.6
- range	1–192

Over the course of time there is a remarkable change in the proportion of males and females, with a major shift to a predominance of female patients. In 1976 Abram reported 61% females; our group in 1985 had 67% females. Veldman et al. (1993) had

76% females, and in this study it is 87.5% (Table XXXI). In spite of this change, there is no change in the predominant age range for CRPS in the last two decades. Pak et al. in 1970 and Kleinert et al. in 1974, reported that 65% of their patients were in the age ranging from 40 to 60 years. In 1985 our patients had a mean age of 41.6 years and Veldman et al. (1993) reported a median age of 42 years. In the present study the mean age was 44.8 years (Table XXXI). The age and gender distribution of patients is shown in Fig.45.

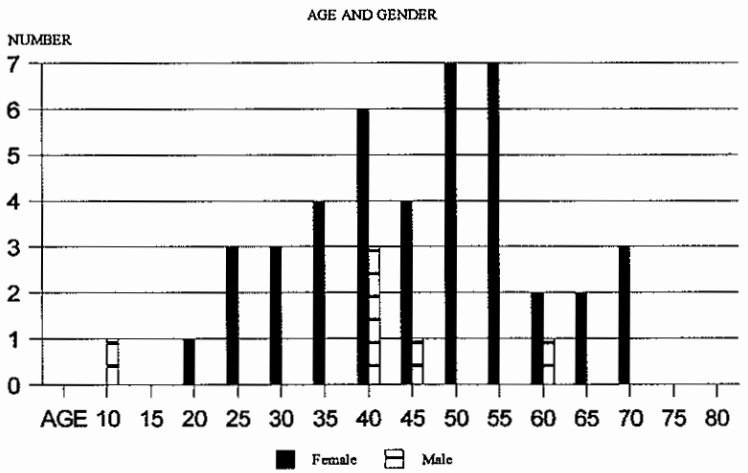

Fig. 45. Distribution of age and gender of the 48 patients in the follow-up study group.

Table XXXII. Origin of CRPS complaints.

	Veldman et al. (1993) n=829	Follow-up study group n=48
Spontaneous	10%	15%
Trauma - blunt	65%	45%
- fracture		15%
- post-operative	19%	23%
- infection		2%

In 1993 Veldman et al. reported that in 10% of their patients no origin of CRPS could be identified. In our group this is 15%. Veldman et al. reported 19% of patients with a post-operative start of CRPS, compared with our 23%. In 60% of our group the onset of symptoms was related to a blunt trauma or a fracture, whereas Veldman and colleagues found this etiological origin in 65% of their patients (see Table XXXII).

Table XXXIII. Location of the CRPS complaints in the study of Veldman et al. and our follow-up study.

	Veldman et al. (1993)	Follow-up study
	n=829	n=48
upper extremity	59%	42%
lower extremity	41%	58%

Concerning distribution of CRPS complaints, Veldman et al. (1993) reported involvement of the upper extremity in 59% and of the lower extremity in 41%. We found a distribution of 42% in the upper extremity and 58% in the lower extremity (Table XXXIII).

The diagnosis CRPS type I was made as before (see Chapter 2, section 2.5, Table V). We asked patients to rate their experienced benefit from the treatment (as a percentage) compared with the complaints experienced before the treatment was started (see appendix A). Symptom scoring was done at initiation of therapy and after three, six and nine months of treatment. Results are shown in Table XXXIV.

Table XXXIV. Percentage of remaining complaints. The complaints at initiation of treatment were taken as 100% (baseline).

patient	3 months	6 months	9 months
1	40%	40%	40%
2	30%	30%	30%
3	10%	0%	0%
4	50%	50%	50%
5	30%	30%	30%
6	60%	50%	40%
7	50%	30%	10%
8	20%	10%	10%
9	10%	5%	0%
10	80%	60%	30%
11	80%	80%	80%
12	20%	20%	20%
13	20%	10%	10%
14	10%	10%	10%
15	20%	20%	20%
16	10%	0%	0%
17	10%	0%	0%
18	60%	60%	60%
19	100%	100%	100%
20	20%	10%	5%
21	20%	10%	5%
22	10%	10%	10%
23	80%	80%	80%
24	10%	10%	10%

25	10%	10%	10%
26	40%	40%	40%
27	20%	20%	20%
28	20%	0%	0%
29	60%	60%	60%
30	30%	0%	0%
31	40%	40%	40%
32	60%	20%	20%
33	40%	40%	40%
34	40%	20%	20%
35	80%	60%	40%
36	10%	10%	10%
37	80%	80%	80%
38	30%	5%	0%
39	30%	20%	10%
40	60%	40%	30%
41	20%	20%	20%
42	80%	80%	60%
43	40%	5%	5%
44	20%	0%	0%
45	20%	10%	10%
46	40%	10%	10%
47	30%	10%	10%
48	20%	20%	20%
mean±SD	36.9±24.6	28.0±26.3	25.3±25.1

Table XXXIV shows the results of scoring of the remaining complaints as a percentage of the original total number of complaints, which was designated 100%. It is remarkable that the most emphatic decrease in complaints was achieved in the first three months of treatment. Patients who did not have a positive reaction during the first three months appeared to have increasing difficulty in achieving a beneficial response during the subsequent 6 months. The optimum result after 3 months was already achieved in 23 (48%) of the patients, with no change to a better result. In 17 (34%) patients the optimum result was achieved in 6 months. Another 9 (18%) patients reached their optimum result after 9 months.

Figure 46 shows that after the first three months there was a large decrease in complaints of 63% (SD ± 24%); between 3 and 6 months there was an increase of 9% success giving 72% (SD ± 25%) reduction in complaints; and between 6 and 9 months there was only a 3% increase of success. And, after 9 months there was an overall mean decrease in the total number of complaints of 75% (SD ± 25%) in the 48 patients.

Similar to the results in the pilot study, the symptoms hyperhidrosis and edema disappeared soon after the ketanserin/carnitine treatment. Because symptoms such as trophic changes, impaired mobility and pain during exercise can be attributed to the underlying pathophysiology, we concentrated the evaluation on the abnormal circulation and the specific allodynic pain. The rationale for this policy was also based on our experience that when we can cure these two essential key symptoms the prognosis for

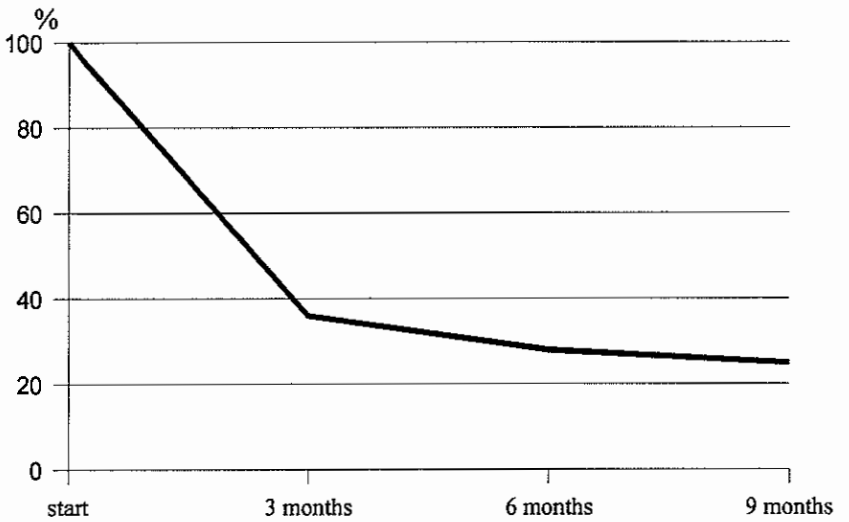

Fig. 46. Mean overall decrease in complaints of the 48 patients in the follow-up study group.

recovery from the disease is very good. Probably, abnormal circulation and abnormal pain are basic pathophysiological phenomena that maintain CRPS.

Treatment of subgroups.

In order to achieve optimal results in the treatment of CRPS patients we decided to intensify treatment. In patients in whom there was no favorable response we continued the intravenous treatment every two weeks. In this way after 9 months we could identify three groups (see Table XXXV). One group of 9 patients (19%) no longer used any medication; most of them were cured or had minimal symptoms which were mainly due to an incompletely healed anatomic fracture, but in all these patients the pain had completely disappeared. A second group of 25 patients (52%) were still receiving oral medication because their clinical status was not yet stable. They had an average of 85% decrease of CRPS complaints after 9 months. The third group of 14 patients (29%) still wanted infusion therapy every two weeks; we continued these infusions because they still produced a positive effect on the plethysmogram, skin temperature recordings and pulse oximetry.

Table XXXV. Division into subgroups 9 months after treatment.

	No medication	**Still receiving oral medication**	**Still receiving infusion therapy**
Number	9 (19%)	25 (52%)	14 (29%)
Age in years (mean)	49.6 (54.8*)	46.7	41.0
Delay in months (mean)	5.2	22.6	9.6
Mean remaining complaints	8%	15%	53%

* see text below

It is important to consider the age of the patients in these subgroups. The mean age of the patients without medication was 49.6 years, which includes a young boy aged 8 years. When we omit this young boy, the mean age increases to 54.8 years (see Table XXXV*). The mean age of the group still using oral medication was 46.7 years, and the infusion group had a mean age of 41.0 years. This suggested the possibility of an inverse relationship between age and curability of CRPS, or of an age-related factor in the pathophysiology of CRPS. In the case of a delay of only a few months between the onset of the CRPS complaints and the start of the treatment this trend was not present.

The mean delay of the group which no longer needed medication was 5.2 months, that in the group on oral medication was 22.6 months, and that in the group which still needed infusion was 9.6 months (Table XXXV).

12.3 Statistical analysis of relationship between age, delay, skin temperature, allodynia and treatment success

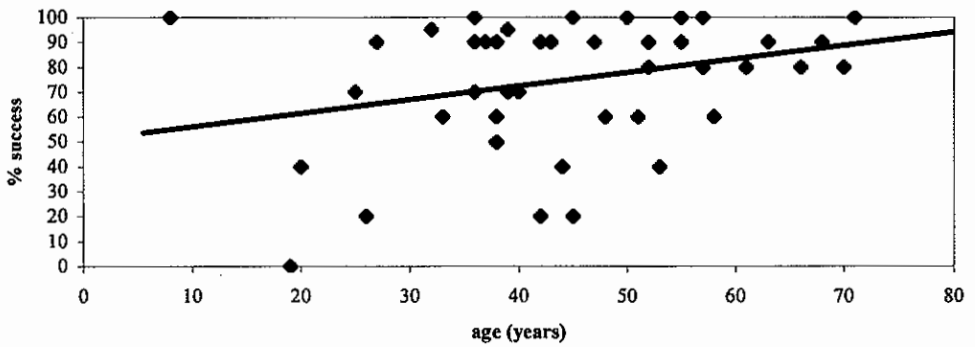

Fig. 47. Relationship between age and success of treatment in 48 CRPS patients.

Because we had the impression that age was an important factor in the curability of CRPS, we evaluated this relationship (Fig. 47) by regression analysis. From the statistical analysis we estimated a mean success score of 74.9% (SD 25.1) in our ketanserin/carnitine treatment, which reflects the decrease in % complaints. For the relationship %success and age: %success = [(0.54±0.26) . age] + 50.48±12, with a correlation coefficient r of 0.300. In case of n = 48 there was a positive significant correlation in the relationship between age and success. Statistically, we may state that for the group of patients 9% of the rate of success is correlated with the age of the CRPS patients.

The delay between onset of symptoms and start of treatment also appeared worthy of evaluation. Using regression analysis we came to the formula: %success = [(0.19±0.27). delay] + 72.76±4.74, with a correlation coefficient r of 0.103. So, there is no correlation ($p>0.05$) (n=48). Delay appears not to affect success rate. It is of interest that 20% success or less was only found in 4 patients with a treatment delay of less than 10 months.

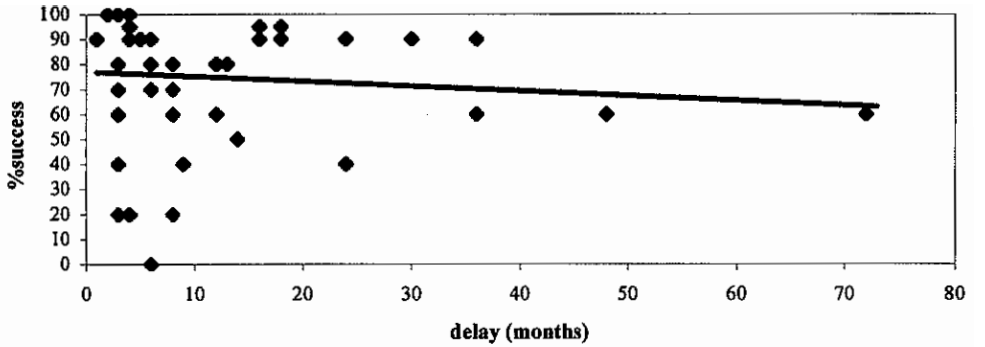

Fig. 48. Relationship between the delay between onset of CRPS symptoms and initiation of treatment, and success of treatment after 9 months.

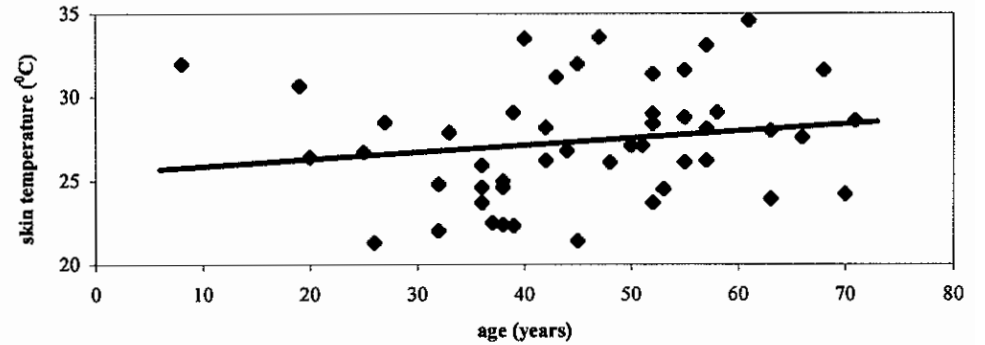

Fig. 49. Relationship between age and skin temperature.

In the subsequent analysis we calculated the relationship between age and skin temperature in CRPS patients. When we consider skin temperature as a parameter of circulation there may be a relationship with age (Fig. 49). The formula for skin temperature was: [(0.64±0.58).age] + 25.2±15.9, with a correlation coefficient r of 0.162.($p>0.05$, n=48).

Next, we calculated whether the trend toward an increasing skin temperature has an influence on the rate of success of treatment (Fig. 50). For different skin temperatures we found the amount of success in the formula: [(0.16±1.06) skin temperature] + (46.0 ± 25.3), with a correlation coefficient r of 0.022 ($p>0.05$, n=48).

Allodynia is probably the most important factor in patients suffering from CRPS. Therefore we evaluated the relationship between this debilitating symptom and other factors. First, in the relationship between the state of allodynia and age we found the formula: [(- 4.13± 1.48) . Allodynia] + 50.95±2.89, r = 0.38 ($p<0.05$, n=48). This is a statistically relevant value. The X-coefficient is negative, so the allodynia is decreasing with increasing age. The implication is that the severity of the allodynic pain is for about 14.5% determined by the age of the CRPS patient (Fig. 51).

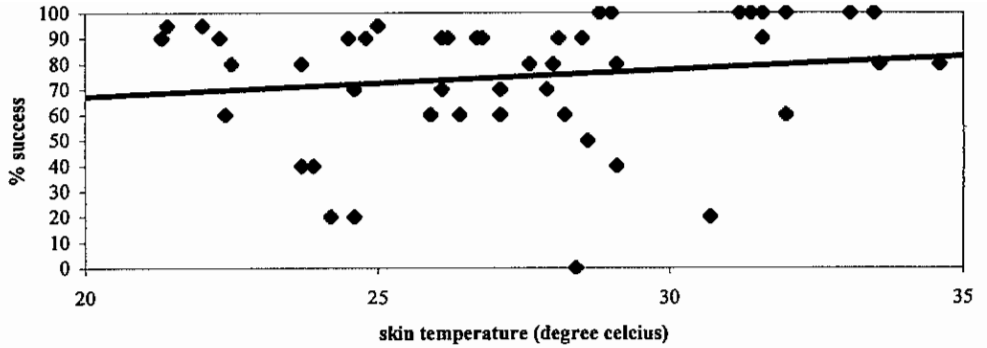

Fig. 50. Relationship between skin temperature and treatment success.

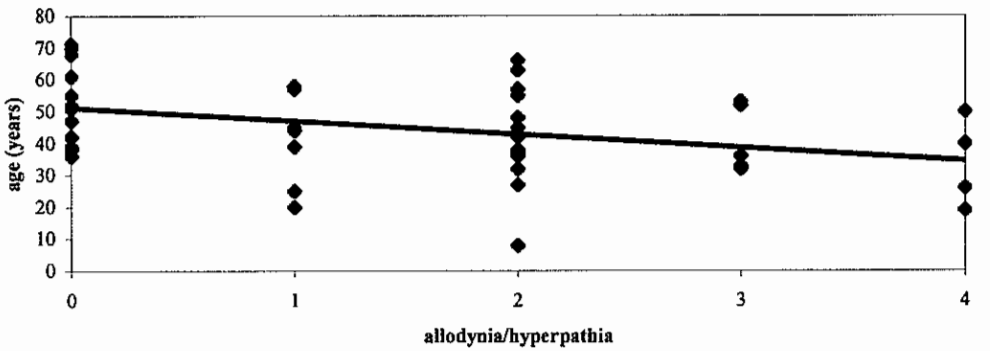

Fig. 51. Relationship between allodynia and age.

Having established a statistical significance between age and the severity of allodynia we then evaluated the statistical relationship between the severity of allodynia and the success of the ketanserin/carnitine treatment. We found in the formula for % success: [(- 9.70±2.53).severity of allodynia] + 89.45±4.95, with a correlation coefficient r of 0.49 ($p<0.05$, n=48). Thus, the severity of allodynia determines the success rate of the treatment for about 24% (Fig. 52).

We then calculated the relationship between skin temperature and allodynia. Regression analysis showed in the formula: stage of allodynia = [(- 0.19±0.40). skin temperature] + 27.61, with a correlation coefficient r of 0.068 ($p>0.05$, n=48) (Fig. 53).

12.4 Summary of results of multiple regression/correlation analysis

The relationship between % success and potential predictors: age, delay, skin temperature and allodynia, in a group of 48 CRPS patients was evaluated, using multiple regression to take account of intercorrelation between predictors. The resulting regression equation

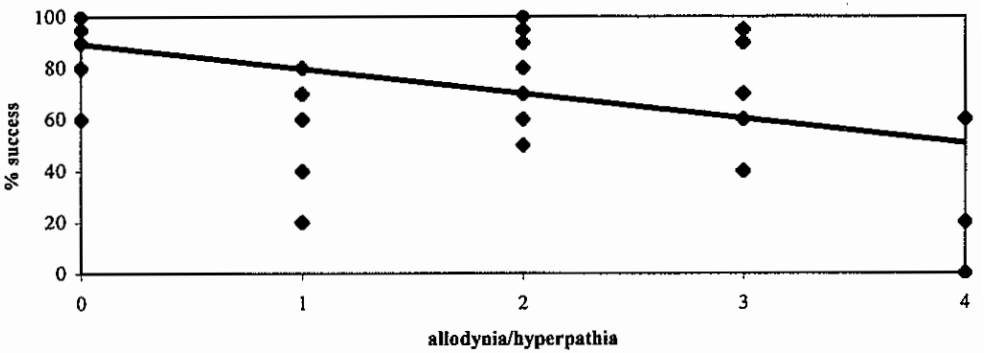

Fig. 52. Relationship between allodynia and success of treatment.

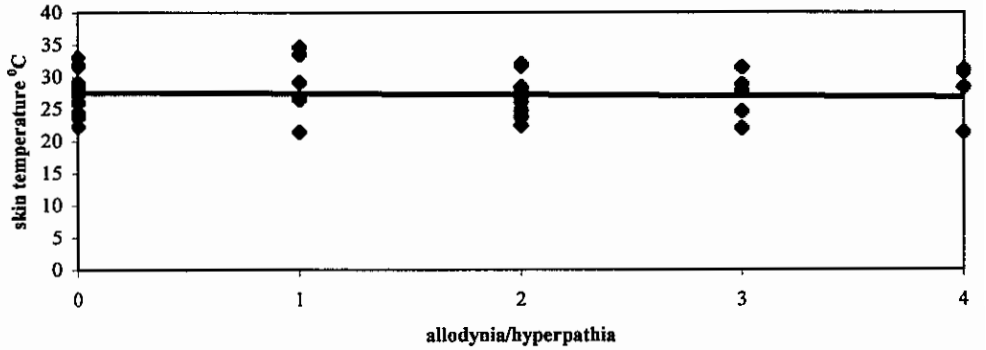

Fig. 53. Relationship between allodynia and skin temperature.

is displayed as follows: % success = (intercept ± SE) + (slope coeff. ± SE) . Age + (idem) . Delay + (idem) . Skin. Temp. + (idem) . Allodynia score; SE = Standard error of Estimate. Plugging in the actual estimates for the respective coefficients in the regression equation, as based on the observations, leads to: %success = (77.2 ± 28.6) + (0.25 ± 0.26) . Age - (8.8 ± 2.8) . Delay - (0.11 ± 0.9) . Skin temp + (0.23 ± 0.24) . Alodynia score.

The "goodnes of fit" of this equation may be judged by the residual deviations between "observed" and "predicted" values, expressed as a Standard Deviation (SD-res.), which is found to be 22.3% (on the scale of % success), or as its square, the residual variance (= 499.1).

A related yardstick of goodness of fit is the coefficient of determination, which is given by the expression: 1- (residual variance / total variance) . (df_{res} / df_{tot}), where 'total variance' stands for the variability between patients as to % success and df = number of 'degrees of freedom' for residual and 'total variance', respectively. In this case the total variance between patients = 628.2 and thus the coefficient of determination = 1 - (499.1/628.2) . (43/47) = 0.28. This coefficient may be regarded as the equation of

the total variability, that is explained by the 4 factors in the equation taken together, which is therefore 28% in this case. Out our analyses we concluded that from the four factors: age, delay, skin temperature and allodynia, only age and allodynia had a statistically significant relationship with success. Therefore the 28% is the result of the relationship with age, expressed in the equation %success = (50.5±12.0) + (0.546±0.256) . age, which explains 9% of the total variance. The other contribution is made by the relationship of success and allodynia, expressed in the equation %success = (89.5±5.0) + (-9.7±2.5) . allodynia, which explains by itself 24% of the total variance.

12.5 Worst scenario cases

In the last two decades there is an increasing trend that females are the predominant victim of CRPS (87.5%). Although the majority of CRPS patients were aged between 40 and 60 years (65%), the mean age for need of long-term infusion treatment is 41 years. These data indicate that the most seriously threatened group at risk for CRPS is that of the younger females. The general description of CRPS in these cases is: one extremity with edema, generally extremely cold skin, often cyanotic and extremely painful, the patients do not want the skin of their affected limb to be touched. Prognosis concerning curability of the disease is mainly dependent on the severity of the allodynic pain. Other factors such as skin temperature and delay before treatment are not decisive parameters in the prognosis of CRPS. The five worst cases found in this follow-up study were the following:

- a 14-year-old girl had osteochondritis dissecans lateral femural condyle. There were no complaints after the first operation. A fall necessitated a second operation. One week after this re-operation she suffered onset of severe CRPS. Treatment with mannitol and n-acetylcysteine had no effect. In addition to the CRPS affecting the left foot, after an infusion in the right arm, CRPS affected that arm too. She is still in treatment for over one year now, receiving ketanserin and carnitine infusions. The edema is gone, but a skin temperature of 27 °C and severe allodynia persist.
- a 26-year-old woman had much pain after a nail excision of the left foot. Several additional operations were performed before CRPS was diagnosed. DMSO application had no effect, mannitol infusions had no effect. After infusion she also developed a mild CRPS on the infused arm. She has been receiving ketanserin/carnitine medication for more than one year. The arm is almost healed, the foot remains problematic, she cannot walk, but she has only mild complaints at rest. This patient also has hyperthyroidism.
- a 41-year-old woman had a contusion of the foot, requiring physiotherapy for many years, CRPS, with skin temperature of 30.2 °C and severe allodynia. After 1.5 years of oral medication and infusion therapy there are mild remaining complaints, but she is now able to walk. This patient also has severe diabetes.
- a 20-year-old woman had a contusion of the left wrist, severe CRPS, and received physiotherapy for many years. She suffered from a left wrist fracture. After immobilisation the CRPS was extremely severe, especially the allodynia. After 1.5 years of oral medication and infusion therapy with ketanserin and carnitine (and psychological help) the left arm is stable but unfunctional and remains in a brace.

12.6 Conclusions

Table XXXVI presents an overview of the statistical analyses on the relationships between the factors age, delay between onset of the symptoms and start of treatment, skin temperature, allodynia and treatment success.

Table XXXVI. Summary of statistical analyses on epidemiological factors and treatment success, and the clinical manifestations skin temperature and allodynia in 48 patients.

	r- values	Level of significance
Age and success	0.300	$p<0.05$
Delay and success	0.103	$p>0.05$
Skin temperature and age	0.162	$p>0.05$
Skin temperature and success	0.022	$p>0.05$
Allodynia and age	0.381	$p<0.05$
Allodynia and success	0.492	$p<0.05$
Allodynia and skin temperature	0.068	$p>0.05$

Remarkably, there was a significant relationship between the factors age and treatment success, and between allodynia and age. In addition, the relationship between allodynia and treatment success was also significant. In contrast, the relationships between delay and success, skin temperature and success and between allodynia and skin temperature were not significant. On theoretical grounds we had expected a significant relationship between skin temperature and age, but this was not the case. In this study, in the overall analysis of CRPS, allodynia proved to be a focal point of the disease. Circulation disorders are closely related, but are probably included in the reaction of the organism and may explain the mechanism whereby the complaints of allodynia persist. The remaining symptoms, such as hyperhidrosis, trophic tissue changes, disturbances in nail growth, shiny skin and coarsening of the skin are secondary phenomena which are not decisive for the pathophysiological course of CRPS.

Chapter 13 GENERAL DISCUSSION

13.1 Characteristic features of CRPS over the years

For decades, clinicians have been discussing both the definition and symptomatology of CRPS (see Chapters 1 and 2). In the present study we used seven symptoms (Chapter 2, Table V) to characterize the disease. Comparison of the number of symptoms found in a group of 45 patients evaluated more than 10 years ago with those of a group of 48 patients investigated the last two years (see Chapter 7, Fig. 17) (Chapter 13, Fig. 54),

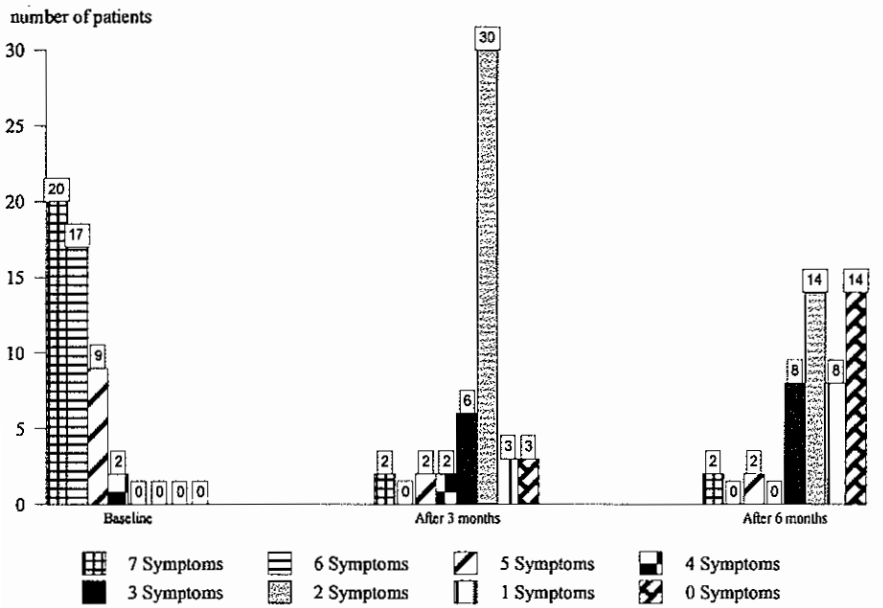

Fig. 54. Total number of symptoms scored in 48 CRPS patients treated with ketanserin plus carnitine (Chapter 12).

shows that in the earlier group 22 of the 45 patients (49%), had 5 or more symptoms, compared with 46 of 48 patients (98%) in our later group. This difference indicates that the severity of the disease has increased in our patients during the last 10 years. This is both remarkable and disturbing, bearing in mind that nowadays CRPS is much better understood and documented than in the past. When we compare the qualitative inventory of the seven symptoms (see Chapter 7, Fig. 20 and Chapter 13, Fig. 55) there is a difference between the three symptoms: pain at rest, pain on movement, and impaired mobility. In the earlier 45 patients this symptomatology was present in 64% of the group, compared with being present in 99% of the more recent 48 patients. There is a smaller difference in the presence of abnormal skin temperature (an indicator of circulatory flow disturbance), namely 69% in the former group and 81% in the latter group. The

symptoms, suggesting derangement of the sympathetic nervous system, i.e. edema and hyperhidrosis, also show a large difference, namely 42% in the former group and 85% in the latter group. The specific symptoms of hyperpathia and/or allodynia show very little difference, 33% and 31%, respectively. These comparisons are summarized in Table XXXVII.

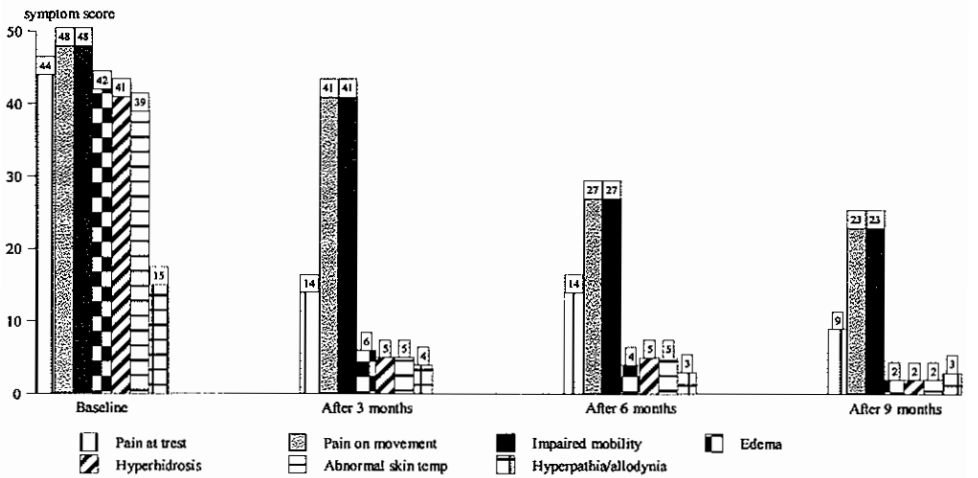

Fig. 55. Trends in symptomatology in 48 CRPS patients (Chapter 12).

Table XXXVII. Symptomatology of patients in the ketanserin monotherapy study and the later ketanserin plus carnitine study.

Year of arrival of the patient	1982–1984	1996–1998
Number of patients	45	48
Number of patients with 5–7 symptoms	49%	98%
Patients with pain at rest and movement, and impaired mobility	64%	99%
Patients with abnormal skin temperature	69%	81%
Patients with edema and hyperhidrosis	42%	85%
Patients with hyperpathia/allodynia	33%	31%

In daily practice we already had the impression that the disease tended to worsen over the years. This was the main reason to intensify the treatment by means of repeated infusion therapy (Chapter 12). Thus, the severity of the disease is increasing and, as mentioned before, more women are being affected than men.

13.2 Comparison between efficacy of ketanserin alone and ketanserin plus carnitine therapy

A complicating factor is that CRPS is a dynamic pain syndrome, which progresses through several stages. As a consequence, not every treatment may be effective at every

stage. This may explain why results from different studies are difficult to reproduce: for example, the results may be from a group of CRPS patients in stage I of the disease and the same treatment may have totally different results (often very poor) in stage II or stage III (Kingery 1997). Other important factors with regard to the efficacy of treatment are the age and gender of the patient (Chapter 12).

In Chapter 7 we showed that the ketanserin monotherapy resulted in 11 (24%) patients being free from disabling complaints after 3 months, and 26 (60%) patients after 6 months. Results in patients receiving the combination therapy, (ketanserin plus carnitine) are shown in Tables XXXIV and XXXV (Chapter 12). As can be seen, after 3 months 27 (56%), after 6 months 32 (67%) and after 9 months 34 (72%) patients were without disabling complaints. Results for both groups are summarized below in Table XXXVIII.

Table XXXVIII. Number of patients without disabling complaints following ketanserin monotherapy, and after ketanserin plus carnitine therapy. * Of these patients 14 had stopped medication. ** These patients showed $\leq$ 30% remaining complaints.

	after 3 months	after 6 months	after 9 months
Ketanserin alone (n=45)	11 (24%)	26 (60%)*	
Ketanserin plus carnitine (n=48)	27 (56%)**	32 (67%)**	34(72%)**

It is interesting that the results after 3 months of combination therapy are considerably better than after ketanserin monotheapy, despite the fact that symptoms in patients receiving the combination therapy were more severe. It is also evident that it is worthwhile to continue combination therapy for at least 9 months. Experience learned that for severe cases it is advisable to continue treatment for even longer periods. Our policy is to continue intravenous treatment for as long as the monitoring shows a beneficial effect as measured by an increase in skin temperature ($>$ 3 °C) and a widening of the plethysmogram (3 to 4 times the baseline measurement of the patient).

13.3 Mechanism of action of ketanserin in CRPS patients

Studying the pathophysiological background of CRPS to find some characteristic elements which may lead to effective treatment, two main streams can be distinguished. One neurophysiological viewpoint (see Chapter 4) is that the vicious circle goes from the peripheral nociceptors to the spinal cord and then back to the peripheral pathophysiological conditions via the sympathetic efferent innervation. Another neurophysiological viewpoint is that of Roberts (1986), who proposed low threshold mechanoreceptors as mediators which start the vicious circle leading to CRPS.

As early as 1930, reports appeared showing that blocking the sympathetic innervation of the affected extremity is an important tool in the treatment of CRPS (Spurling, 1930). This phenomenon is used as evidence of the involvement of the sympathetic nervous system in CRPS. Despite the fact that on theoretical grounds the assumption has been made that the sympathetic nerves are involved in CRPS, which led to the development

of treatments which block the sympathetic nerves, such neurophysiological involvement has never been proven.

Based on the symptomatology of changes in circulatory flow in CRPS characterized by vasodilatation and vasoconstriction we decided to block the action of serotonin, which can produce both phenomena in the peripheral circulation.

Vasoconstriction is caused by direct activation of the smooth muscle cells or may be due to the amplification of contractile responses to other antagonists (Chapter 6). Vasodilatation is primarily mediated by the endothelial cells or can be due to inhibition of sympathetic neurotransmission. A disturbance in the balance between the vasoconstrictor and vasodilatator effects can lead to a pathological condition, such as CRPS. Thus, vasodilatation or vasoconstriction may occur in CRPS patients. Potent direct antagonism of the effect of serotonin on blood vessels appears to require high affinity binding to 5-HT_{2A} receptors. Ketanserin, is a drug with strong 5-HT_{2A}-receptor blocking properties (Chapter 6).

13.4 Mechanism of action of carnitine in CRPS patients

There are several ways by which carnitine may act in alleviating the CRPS (see Chapter 8)

- stimulation of the mitochondrial fatty acid oxidation,
- stimulation of the pyruvate oxidation, which decreases the production of lactic acid,
- stimulation of the oxidation of 2-ketoglutarate and branched-chain amino acids,
- conversion of excessive long-chain acyl-CoA into acylcarnitine, removing metabolic inhibition and preventing production of free radicals,
- improvement of the microcirculation in ischemia by repletion of interstitial carnitine which exchanges with LCAC from cells,
- membrane repair by reacylation of peroxidised fatty acyl groups in phospholipids.

In Chapter 10 (Figs. 27 and 28) we showed the age-dependent level of TC and FC in 8 female controls. In Chapter 11 (Figs. 38 and 39) we showed similar results in 45 female CRPS patients. The regression lines of the two groups are, however, not parallel. Figures 56 and 57 show that the levels of TC and FC in the CRPS patients increase with age, but less than in controls. In young CRPS patients the levels are higher than in controls, while at old age the levels are similar.

In relation to age, the levels of TC and FC in CRPS patients increase at a much slower rate than in the controls. The regression lines cross at age 73 years for TC and at age 65 years for FC. As cited in Chapter 8, the precise plasma level in an individual is determined by the difference between input (renal reabsorption, intestinal transport, renal and hepatic biosynthesis) and output (uptake by muscle, heart and other cells) (Scholte et al. 1996). It is likely that this balance is changed in the (younger) CRPS patients, compared with controls, by a lower output. The carnitine uptake in the ischemic area is decreased, both by lowered perfusion and by inhibition of the carnitine transporter by the increased LCAC (Chapter 8, section 8.4) and there may be a contribution by the release of carnitine from the endothelial cells (Hülsmann et al. 1996).

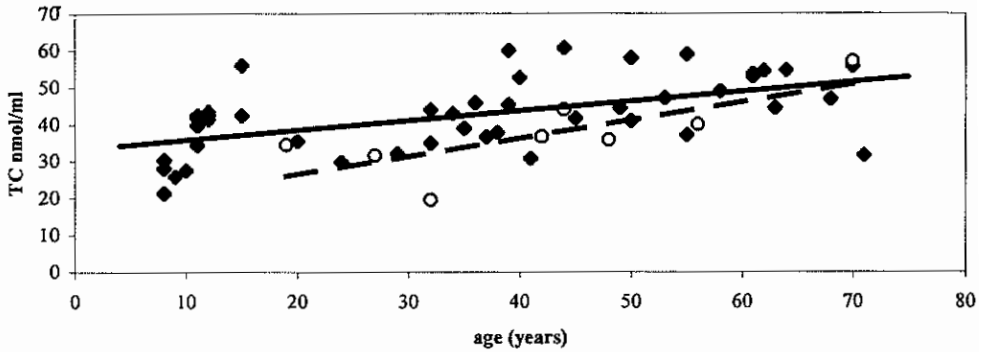

Fig. 56. TC levels of 8 controls (open dots) and of 45 female CRPS patients (diamonds), with regression lines. Dashed line is the regression line of controls, solid line is the regression line of CRPS patients.

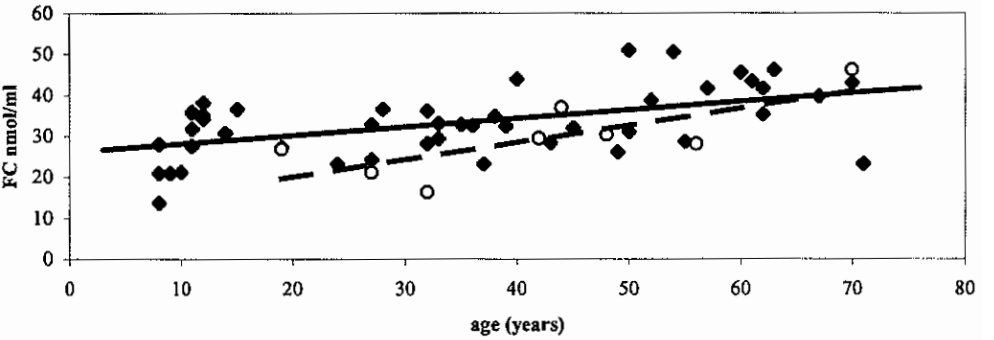

Fig. 57. FC level in 8 controls (open dots) and of 45 female CRPS patients (diamonds), with regression lines. Dashed line is the regression line of controls, solid line the regression line of CRPS patients.

As shown in Chapter 10 (Table XX) 1 hour after 1 g i.v. carnitine there is an increase of TC (633%), FC (776%), C2-C (130%) and C3-C (148%). For the pharmacodynamic profiles in the first hour see Chapter 10 (Figs. 33 and 34). Peak levels of FC and TC are generally reached rapidly, within a few minutes, while the level of C2-C is still increasing after 1 hour.

13.5 Characteristic features in therapy responsive and therapy resistant patients, and in patients with a relapse

CRPS is a complex disease with a broad scala of clinical manifestations. There are mild cases with few symptoms limited to one extremity, sometimes with a spontaneous remission, and otherwise easy to cure. On the other hand, there are seriously debilitating conditions with severe symptoms affecting more than one extremity (sometimes the

entire body) and almost impossible to cure. The symptoms can be mild and stable, or can progress in severity over time. Therefore, the pathogenesis of CRPS is highly complex and incorporates the possibility to aggravate the condition itself.

The characteristic symptoms, abnormal pain and circulatory disorder, indicate involvement of a peripheral micro-anatomical complex of different cell types. From the nervous system side sensori-motor and sympathetic nerve endings are involved and from the circulatory side endothelium, smooth muscle and skeletal muscle. These neural and circulatory components will influence each other in a variable way. Therefore the pathogenesis of CRPS probably involves several components which can act independently, but also interact with each other.

We found in healthy females that the level of plasma TC and FC is age-related (see Chapter 10, Figs. 27 and 28) and that ketanserin affects the TC and FC levels up to the age of 42 years (see Chapter 10, Figs. 29 and 30).

With increasing age of CRPS patients, the hyperpathic and/or allodynic pain decreases (see Chapter 12, Fig. 51). The susceptibility for catecholamines also decreases with age. Therefore, carnitine supplementation probably influences the CRPS pathophysiology in different ways at different ages. As shown in Table XXXV (Chapter 12), patients benefitting most from treatment have a higher age. An advantage of the infusion and oral therapy is that we never have to physically handle the diseased extremity; moreover, the strong vasodilatating effect gives the patient a positive sensation. These factors might explain why no worsening of the symptoms was observed during therapy (Chapter 12, Table XXXIV).

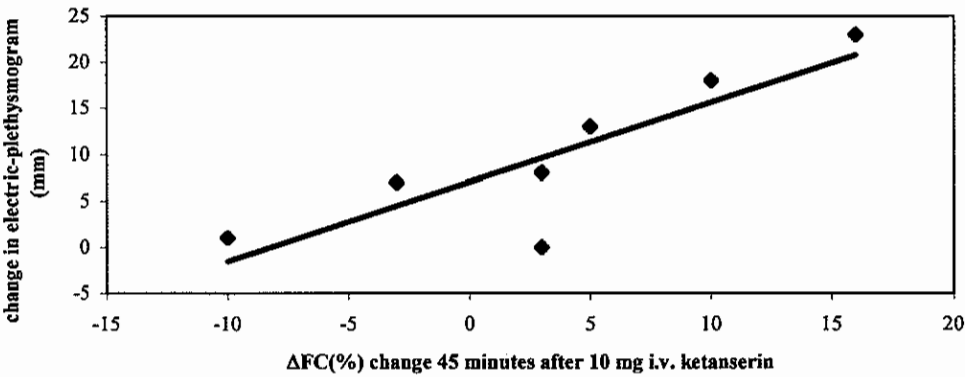

Fig. 58. Relationship between the FC(%) and the change in amplitude on the plethysmogram.

When comparing the effect of ketanserin on the plasma TC and FC levels in controls (Chapter 10, Figs. 29 and 30), and CRPS patients (Fig. 43) it is obvious that not every CRPS patient reacted in the same way. For example, a female patient aged 20 years, who was expected to have a rise in plasma FC level after ketanserin, had a marked decrease (-10%). This result might indicate the severity of the CRPS, because patients with severe CRPS show little or no effect on the plethysmographic trend recording at the start of intravenous treatment. Thus, in severe cases of CRPS ketanserin may be unable to increase the circulatory flow. Therefore, we compared the clinical parameters of the trend monitoring, (i.e. changes in plethysmography, skin temperature and pulse

oximetry), with the changes in plasma FC levels 45 minutes after 10 mg i.v. ketanserin (Fig. 58).

The relationship between the percentage change in FC level and the change in the plethysmographic recording 45 minutes after 10 mg i.v. ketanserin was significant ($r=0.85$, $p<0.01$, $n=7$). The recordings of skin temperature and pulse oximetry were not correlated, but these parameters may be influenced less by ketanserin than by other regulatory mechanisms.

In those patients with more than 3 months treatment we compared the rate of success with the amplitude change in the plethysmographic trend recording (Fig. 59). There was a significant correlation between the rate of success and the plethysmographic change ($r=0.74$, $p<0.05$, $n=9$); this is in accordance with our clinical experience. When a CRPS patient is recovering, the baseline temperature normalizes and the amplitude change after ketanserin decreases. When there is no (or minimal) change in these parameters we stop the intravenous treatment and continue with oral treatment only.

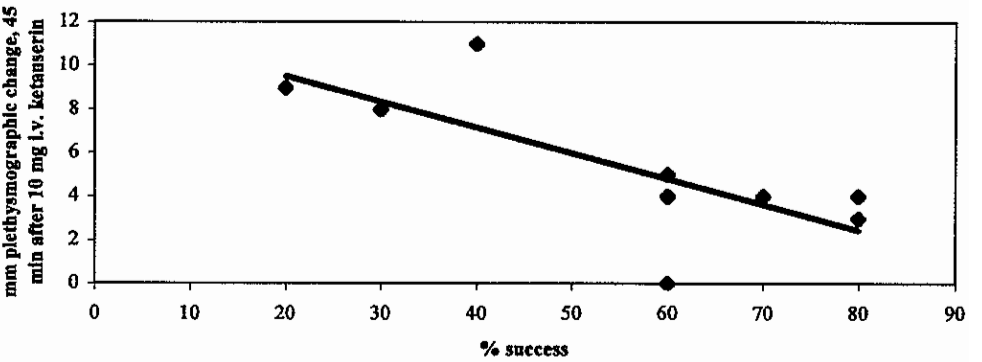

Fig. 59. Rate of success in a later stage of treatment related to the change in amplitude on the plethysmogram (mm).

Thus, at the start of treatment the change in plasma FC level and the change in the plethysmogram 45 minutes after 10 mg i.v. ketanserin indicate the severity of CRPS. The diminishing change in plethysmographic amplitude in a later stage of treatment is an indication of the effectiveness of the treatment.

Patients may suffer from CRPS on more than one occasion. We have treated five patients who developed CRPS for a second time, each time after a second mild trauma. In three patients the same extremity was affected, whereas in the others it was a different extremity. All five were women aged between 25 and 45 years. In two cases we were able to estimate the change in FC level 45 minutes after 10 mg i.v. ketanserin; despite the fact that both patients were aged < 44 years, (23 and 37 years), both showed a decrease in FC levels (-5(%) and -4(%), respectively).

When surgery is necessary on a CRPS extremity in our clinic, it is performed with a continuous ketanserin infusion. The day before operation we administer ketanserin at 4 mg/h which is continued for at least 48 hours postoperatively. To date, these operations have been successfully performed without relapses.

13.6 Concept of pathophysiology of CRPS

Our research led to a focus on two related areas involved in the pathophysiology of CRPS. One is the regionally disturbed circulatory flow, and the other is the regionally disturbed cellular metabolism. Normally, the body can handle temporary local ischemia and the subsequent phase of reperfusion, but in CRPS a mechanism is triggered, whereby the disturbed metabolism causes a further decrease in circulatory regional flow and a circulus viciosus arises.

In the pathogenesis of the disturbed local circulatory flow, serotonin and 5-HT_{2A} receptors play an important role (Chapter 6). Ketanserin has a high affinity with these receptors and is effective in relieving the circulatory flow disorder. Relieving the circulatory flow dysfunction was, thus, an important step in the treatment of our CRPS patients (Chapter 7).

The mechanism by which a regional disturbed circulatory flow will lead to CRPS is not yet known.

The FC change after ketanserin infusion in the CRPS patients is correlated with the widening of the plethysmogram, and that is a measure for the perfusion. How this increased perfusion gives rise to an increase of the FC levels of the CRPS patient, who already has increased FC levels compared with controls, is puzzling. It is not likely that it is caused by the exchange of carnitine with acylcarnitine from the ischemic area, and further metabolism of the acylcarnitine in the non-ischemic part of the circulation, because it is not expected that carnitine releases more acylcarnitine on a molar basis. It is on the other hand, well possible, that the reperfusion of the ischemic area costs more carnitine than it pays to the circulation. If so, the increased perfusion releases carnitine from another part of the body. As the improvement of the flow is systemic, this must give rise to increased hepatic and renal biosynthesis of carnitine, increased renal reabsorption and/or increased transport from the intestine to the blood. Further research of this process is tempting, as it may be closely related to the pathophysiology of CRPS.

13.7 Final remarks (future developments)

In case of severe regional ischemia, as in CRPS, the normal protective mechanisms of the organism fail. With the serotonin-antagonist ketanserin, a major part of the vasoconstriction can be relieved. Additional carnitine infusion gives a second vasodilatory effect which leads to a simultaneous increase in adenosine and ATP plasma levels, Capecchi et al. (1997) showed that adenosine peaks before ATP. There is no evidence for a lack of ATP and/or adenosine; in fact, adenosine, generated by enzymatic degradation of ATP, is enhanced during ischemia. Adenosine inhibits norepinephrine release from sympathetic nerve endings, causing vasodilatation via endothelium-dependent and endothelium-independent actions. Ralevic and Burnstock (1996) clearly demonstrated the relative contribution of P_{2u} and P_{2y} purinoceptors to endothelium-dependent vasodilatation in isolated mesenteric arterial bed from the golden hamster. When the endothelium was removed they found that the vasoconstrictor responses to ATP and norepinephrine were augmented. Their results showed that ATP elicits vasoconstriction of mesenteric arteries of the golden hamster via P_{2x} purinoceptors located on the smooth muscle, and vasodi-

latation via P_{2u} purinoceptors which are located on the endothelium. P_{2y} purinoceptors contribute minimally to relaxation to ATP in hamster mesenteric arteries. Burnstock and Wood published in 1996 a more detailed description of purinergic receptors, their role in nociception and primary afferent neurotransmission, and the role of adenosine. The recent discovery of a P_{2x} purinoceptor (a ligand-gated ion channel triggered by ATP) that is selectively expressed by small-diameter sensory neurons has led to the exploration of the sources of ATP involved in the initiation of different types of nociception and pain, including sympathetic nerves, endothelial cells and tumor cells. In addition, the anti-nociceptive actions of adenosine via prejunctional P1 (A1) purinoceptors in the spinal cord and the pain-enhancing actions of adenosine via P1 (A2) purinoceptors in the periphery are very interesting. Bo et al. (1998) showed pharmacological and histochemical evidence for P_{2X} receptors in human umbilical vessels. These P_{2X} purinoceptors were located in the smooth muscle of the human umbilical vessels. Bodin and Burnstock (1995) studied the effects of lipopolysaccharide, a potent inflammatory mediator, on the shear stress stimulated release of ATP from endothelial cells from human umbilical vein in primary culture. Their results showed that undamaged endothelial cells release ATP under these experimental inflammatory conditions and support an early role of extracellular ATP in the inflammatory process. It is possible that a disturbance in the sensitivity of purinoceptors of endothelium and/or smooth muscle is responsible for this debilitating process of vasoconstriction in CRPS patients. Further investigation is needed to clarify the exact mechanism of this pathophysiology in which ATP and adenosine play an important role. The clinical situation is much more complicated, because catecholamines and the sensitivity for catecholamines are also involved.

Drummond et al. (1991) measured the significance of differing plasma catecholamine concentrations in affected and unaffected limbs in CRPS patients. They found lower concentrations of plasma norepinephrine on the painful side in patients with widespread allodynia and in those with hyperhidrosis in the affected hand or foot. These findings indicate that autonomic disturbances, as in CRPS, are not due to sympathetic overactivity. It is more likely that the spontaneous pain and allodynia are the result of supersensitivity to norepinephrine. Drummond (1995) also found that noradrenaline and sympathetic neural activity might increase pain associated with skin damage.

Kurvers et al. (1996c) accepted the view that sympathetic dysfunction in CRPS has been purported to consist of an afferently-induced increase in efferent sympathetic nerve impulses (somato-sympathetic reflex) and/or denervation-induced supersensitivity to catecholamines. Their results suggest that sympathetic dysfunction in extremities of patients with CRPS distal to the site of trauma consists of hypersensitivity to catecholamines at stage II and III as a result of autonomic denervation at stage I, whereas proximal to the site of trauma, sympathetic nerve impulses may be increased at all three stages. In the model of loose ligation of a sciatic nerve in rats, which produced signs and symptoms similar to those observed in human conditions of neuropathic pain, Kurvers et al. (1997a,b) found supersensitivity of skin microvessels to catecholamines. From out the view that denervation-induced supersensitivity to circulating catecholamines has been implicated in sympathetic dysfunction in the chronic constriction injury model in sciatic nerve ligation in rats as well as in CRPS, Kurvers et al. (1998) studied functional properties of sympathetic innervation in subcutaneous resistance arteries, isolated from the hind paw of rats three weeks after ligation. Contractile responses to electric field

stimulation of adrenergic agonists were studied using a myograph. Compared with the contralateral side, subcutaneous arteries from the ligated side were less responsive to electrical field stimulation. Besides, compared with the contralateral side, subcutaneous arteries from the ligated side showed increased sensitivity to " 1-adrenoceptor stimulation. Their study demonstrates that sympathetic dysfunction in an experimental model of neuropathic pain consists of denervation-induced supersensitivity to catecholamines rather than of an afferently-induced increase in efferent sympathetic nerve impulses. Besides these effects on the microcirculation there are other pathophysiological factors building up the whole complex of symptomatology in CRPS. These microcirculatory effects of vasoconstriction and vasodilatation can be monitored by photo-electric plethysmography and skin temperature measurement. The oxygen consumption can be monitored by pulse oximetry. Especially in the delayed state of CRPS, symptoms of allodynic pain as part of neuropathic pain and motor dysfunction may play an important role. In an animal model of neuropathic pain, Daemen et al. (1998a) studied the tissue accumulation of polymorphonuclear leukocytes as phenomena of neurogenic inflammation. They found that loose ligation of a sciatic nerve induces an inflammatory response in the ipsilateral hindpaw, which most likely is mediated by release of neuropeptides from the peripheral endings of antidromically acting nociceptive C-fibers. Drummond et al.(1994) measured the concentrations of neuropeptide Y, a vasoactive transmitter which co-exists with norepinephrine in sympathetic nerve terminals, in the painful and contralateral limbs in patients with CRPS and causalgia. The concentrations of neuropeptide Y were generally lower in the painful limb if it was warmer than the contralateral limb. They suggested that a reduction in sympathetic activity might accompany allodynia and influence vasomotor disturbances in patients with causalgic pain. Evidence was presented by Bullens et al. (1998) that also in the field of motor disturbances, like joint stiffness and tremor, chronic constriction of the sciatic nerve of the rat can be used as an animal model of CRPS. They found a decrease of acetylcholinesterase-positive fibers in cross-sections taken from distal and proximal sciatic nerve biopsies ipsilateral to the ligatures. Motor denervation also affected the contralateral nonligated sciatic nerve. These results are positive about the involvement of the central nervous system in the pathophysiology of CRPS. Daemen et al. (1998b), confirmed the findings of Bullens and colleagues in the same model. They found that a reduction in the number of motor nerves fibers is more pronounced distal to the side of the ligatures than proximal. A less pronounced reduction of motor fibers was observed in the ipsilateral, nonligated femoral nerve. This spread outside the territory of the primarily affected nerve, suggests degeneration of motor neurons at the level of the central nervous system.

Thus, it is expected that future research will focus on drugs which influence the sensitivity of catecholamines (Kurvers et al. 1998), and those that block P_{2x} or P_{2y} receptors (Burnstock 1996a,b, Rongen et al. 1997). Such studies are mainly based on the need to control the allodynic pain component in CRPS and in other neuropathic pain syndromes. Studies on $5HT_{2A}$ receptors have already shown that ketanserin has an anti-allodynic effect (Pierce et al. 1996). Our study demonstrates that ketanserin plus carnitine has a much stronger anti-allodynic effect in CRPS patients than ketanserin alone.

Tissue involved in CRPS is in a state of oxidative stress. The defense mechanisms of the body, as well as ketanserin, will create a state of reperfusion which may be suitable

for studies on the effects of carnitine and carnitine-esters. Endogenously formed low but significant levels of long chain acyl carnitines (LCAC) (see Chapter 8, section 8.4) may restore the deterioration in ischemic areas, and excess LCAC is released by carnitine. Besides these effects in the reperfusion state, direct vasodilatory effects, by propionyl-L-carnitine, as recently demonstrated by Cipolla et al. (1999), may have a beneficial effect on CRPS symptomatology. The efficacy of propionyl-L-carnitine was also shown to be significantly better than carnitine in peripheral arterial disease, another disorder of the microcirculation (Brevetti et al. 1992).

Chapter 14 SUMMARY

For a century, the clinical presentation and the name of the disease nowadays called Complex Regional Pain Syndrome (CRPS) has been a matter of debate: starting with Sudeck's atrophy (1900), followed by various other names, then Sympathetic Reflex Dystrophy (Syndrome) and eventually CRPS. Even though the Taxonomy Committee of the International Association for the Study of Pain, agreed upon this name, in recent years new proposals have emerged to change the name again, along with a new proposal asking for uniformity in the classification of the symptoms to assure a better comparison in research and treatment between groups of patients (Chapter 2).

Although the exact pathophysiology is still not fully elucidated (Chapter 3), circulatory flow disturbances and pain are the most important symptoms of CRPS.

Despite all research conducted in the past, to date there is no consensus about the primary treatment (Chapter 4).

In Chapter 5 objective measurements of CRPS symptoms are introduced and their application in CRPS treatment is presented in Chapter 9. We choose for a setting in which we are able to monitor simultaneously, photoelectric plethysmography, and skin temperature and pulse oximetry. In this way, effects on peripheral circulatory flow changes during infusion therapy can be visualised and effectiveness of therapy recorded.

On theoretical grounds, both ketanserin alone and carnitine alone will have positive effects on the pathophysiological problems in CRPS. Ketanserin, as a 5-HT_{2A} receptor blocker, restores the circulatory flow and has properties that will diminish neuropathic pain (Chapter 6).

Results of ketanserin monotherapy are evaluated in Chapter 7. In a double-blind cross-over study the effect of ketanserin on the peripheral circulatory flow was shown to be statistically significant. A follow-up study demonstrated the beneficial effect of ketanserin on the symptoms of CRPS. The results were promising and, in contrast to other treatments, ketanserin was also effective in patients who had CRPS for a long time.

Carnitine and the acylcarnitine esters play an important role in the metabolism of all cells. The actions of carnitine are diverse and include: 1) stimulation of the mitochondrial oxidation of fatty acids, the 2-ketoacids pyruvate, 2-ketoglutarate and branched-chain 2-ketoacids, 2) the conversion of long-chain acyl-CoA which are patent inhabitors of several enzyme systems, into long-chain acylcarnitine, a process which also decreases the production of free radicals, 3) membrane repair by reacylation of peroxidised fatty acyl groups in phospholipids, 4) stimulation of the microcirculation in ischemia by repletion of interstitial carnitine, which in turn exchanges with long-chain acylcarnitine, and 5) membrane stabilization by a small portion of long-chain acylcarnitine (Chapter 8). This latter theory, which explains the action of carnitine in stimulating the microcirculation in Ca^{2+} overload, was developed by Hülsmann and coworkers and may also be valid for CRPS.

In Chapter 10 we evaluated the normal plasma levels of carnitine and acylcarnitines in healthy women. We discovered that the levels of plasma free and total carnitine

increased with age. Intravenous ketanserin in a younger group (19–42 years) caused an increase in these carnitine levels. This effect decreased with age, and disappeared in the older group (44–70 years). Intravenously administered free carnitine in a dosage of 1 g, gave within a few minutes a 6–8 fold increase in plasma levels of total and free carnitine. Plasma acylcarnitine, mainly consisting of acetylcarnitine with a minor contribution of propionylcarnitine, increased much more slowly. The carnitine household had the ability to restore the age relationship of the free and total plasma carnitine levels in women within 1 hour after the carnitine injection.

In Chapter 11 we showed that the plasma levels of free carnitine, acetylcarnitine and propionylcarnitine in female CRPS patients are higher than the levels found in age-matched controls, and become similar at old age. An age relationship with plasma levels was also present in CRPS patients. Ketanserin intravenous injection led in most patients to an increase in plasma free carnitine level, and this effect appeared to be related to the severity of CRPS.

Chapter 12 presents the results of the combined treatment with ketanserin plus carnitine. A remarkable finding was that over the years more women are affected than men and that the severity of their complaints increases.

Finally, we discuss some elements brought forward by this investigation (Chapter 13). Evaluation of the efficacy of the ketanserin monotherapy compared with that of the ketanserin plus carnitine combination therapy was in favor of the combination therapy, despite the fact that the symptomatology was worse in this latter group of patients. In the combination therapy there was a positive significant relationship between age and treatment success, age and allodynia and therefore between allodynia and treatment success. We discovered in female CRPS patients that after intravenously administered ketanserin, the change in plethysmographic amplitude is related to the change in the plasma free carnitine level.

Further promising directions of research are to investigate whether the ketaniserin plus carnitine combination therapy is also of help to treat other diseases in which a disturbed microcirculation is suspected. It will be of interest to study whether the efficacy of acetylcarnitine and propionylcarnitine is better than that of carnitine. To promote more efficient treatment of the neuropathic pain in CRPS, the development of new analgetics could be of great benefit (Chapter 13).

Hoofdstruk 14 SAMENVATTING

De discussie omtrent de klinische omschrijving en de naam van het ziektebeeld dat tegenwoordig gecompliceerd lokaal pijn syndroom wordt genoemd en in het Engels "Complex Regional Pain Syndrome" (CRPS) is al een eeuw aan de gang. Na Sudeck's atrofie (1900) volgden vele andere benamingen, toen kwam de naam die nu nog veel wordt gebruikt: "Sympatische Reflex Dystrofie (Syndroom)," en tenslotte CRPS (Hoofdstuk 2). Zelfs de laatste jaren worden weer nieuwe voorstellen tot naamsverandering gepubliceerd, ondanks het feit dat de Commissie voor Taxonomie van de "International Association for the Study of Pain" consensus had bereikt over de naam CRPS. Ook worden voorstellen gedaan om te komen tot meer uniformiteit van symptomatologie. Dit laatste is bedoeld om patiëntengroepen te krijgen waarbij de resultaten van onderzoek en behandeling beter te vergelijken zijn.

Tot nu toe is niet bekend hoe het ziektebeeld CRPS precies ontstaat (Hoofdstuk 3). Karakteristieke klinische kenmerken zijn een gestoorde doorbloeding en een heftige abnormale pijn. Ondanks al het onderzoek dat is gedaan, is er geen consensus over de beste behandelmethode (Hoofdstuk 4).

In Hoofdstuk 5 worden de methodes besproken waarmee we op een objectieve manier symptomen van CRPS kunnen registreren. In Hoofdstuk 9 wordt de toepassing hiervan nader toegelicht. Er werd gekozen voor een vorm van registratie, waarbij gelijktijdig en continu het foto-elektrisch plethysmogram, de huidtemperatuur en de zuurstofverzadiging van het bloed worden gemeten. Met deze methode kan op een objectieve manier verandering van de perifere doorbloeding worden geregistreerd. Door deze vorm van registratie bij elke behandeling toe te passen, wordt inzicht verworven in de effecten van de medicatie in de acute fase en op langere termijn.Op grond van theoretische overwegingen hebben zowel ketanserine als carnitine eigenschappen die de klinische problemen bij CRPS kunnen laten afnemen. Ketanserine bezet specifiek de 5-HT_{2A} receptoren in de perifere vaatwand, waardoor de perifere doorbloeding verbetert en de neuropatische pijn vermindert (Hoofdstuk 6).

De resultaten van de behandeling met ketanserine worden besproken in Hoofdstuk 7. Een dubbel geblindeerde, "cross-over" studie toonde aan dat ketanserine een statistisch significant effect heeft op de perifere bloeddoorstroming. In een verder onderzoek werd het effect van ketanserine op de CRPS symptomen nagegaan. De resultaten waren bemoedigend, en in tegenstelling tot andere gepubliceerde behandelingsmethoden, bleek ketanserine ook effectief te zijn bij patiënten die al een lange tijd CRPS hadden.

Carnitine en de acylcarnitine esters spelen een belangrijke rol in de stofwisseling van alle cellen. Carnitine heeft vele effecten. Het stimuleert de oxidatie van vetzuren, de 2-ketozuren pyruvaat, 2-ketoglutaraat en vertaktketen 2-ketozuren door mitochondrin. Het zet de lang-keten acyl-CoA esters, die enerzijds belangrijke energiebronnen zijn en anderzijds verschillende enzyme systemen remmen, om in langketen acylcarnitines, een proces dat ook leidt tot een verminderde productie van vrije radicalen. Het kan membranen herstellen door reacylering van geperoxydeerde vetzuren in fosfolipiden. Het stimuleert de microcirculatie in ischemie door aanvulling van het interstitiële carnitine,

dat kan uitwisselen met langketen acylcarnitine, terwijl membranen gestabiliseerd worden door een kleine hoeveelheid langketen acylcarnitine (Hoofdstuk 8). Dit mechanisme waardoor carnitine in staat is om de weefselperfusie te stimuleren bij hoog intracellulair Ca^{2+} dat door Hülsmann en medewerkers werd bedacht en experimenteel onderbouwd, is ook van belang voor het begrijpen van de werkzaamheid van carnitine bij CRPS.

In hoofdstuk 10 worden de normaalwaarden in plasma van carnitine en van acylcarnitines bij gezonde vrouwen gepresenteerd. We ontdekten dat de plasmaspiegel van vrij en totaal carnitine toeneemt met het stijgen van de leeftijd. In de leeftijdsgroep van 19–42 jaar veroorzaakte intraveneus ketanserine een verhoging van deze spiegels. Dit effect nam af met het stijgen van de leeftijd en was verdwenen bij de leeftijdsgroep van 44–70 jaar. Intraveneus carnitine in een dosering van 1 g gaf binnen enkele minuten een 6–8 voudige plasmaspiegel verhoging van vrij en totaal carnitine. Plasma acylcarnitine, dat vooral bestaat uit acetylcarnitine en een geringe hoeveelheid propionylcarnitine, stijgt veel langzamer. De carnitine-huishouding bleek in staat te zijn om na intraveneus 1 g carnitine, binnen een tijdsspanne van 1 uur, de leeftijdsafhankelijke rangorde in de plasmaspiegels van vrij en totaal carnitine te herstellen.

In Hoofdstuk 11 wordt een overzicht gegeven van de carnitine plasmawaarden gevonden bij de vrouwelijke CRPS patiënten. Het bleek dat de plasmaspiegels van vrij carnitine, acetylcarnitine en propionylcarnitine hoger liggen dan bij gezonde vrouwen van dezelfde leeftijd. Op hogere leeftijd worden ze gelijk. Zoals bij controles bestaat er bij de CRPS patiënten een relatie tussen leeftijd en plasmaspiegels, en heeft intraveneus ketanserine een effect op de FC plasma concentratie. Dit laatste effect wordt mede bepaald door de ernst van de ziekte.

In Hoofdstuk 12 worden de behandelresultaten beschreven van carnitine in combinatie met ketanserine. Opvallend is dat de symptomatologie van CRPS in de laatste jaren verergerd is en dat het aantal vrouwelijke patiënten fors is toegenomen in verhouding tot het aantal mannelijke patiënten.

In Hoofdstuk 13 worden bevindingen van dit onderzoek besproken. De behandelresultaten van de ketanserine monotherapie worden vergeleken met die van de ketanserine plus carnitine combinatietherapie. Ondanks het feit dat de symptomatologie van de patiënten, behandeld met ketanserine plus carnitine, ernstiger is dan die van de groep behandeld met alleen ketanserine, zijn de behandelresultaten beter. Deze vertonen een statistisch significante positieve correlatie met het stijgen van de leeftijd en de ernst van de allodynia, zodat er ook een relatie is tussen allodynia en behandelsucces. We ontdekten in de vrouwelijke CRPS patiënten dat na intraveneus ketanserine, de veranderingen van de amplitude van het plethysmogram gecorreleerd bleek te zijn met de veranderingen van de plasma vrije carnitine concentratie.

Toekomstige veelbelovende richtingen van onderzoek zijn het nagaan of de combinatietherapie ook werkzaam is bij ziektebeelden waarbij ook sprake is van gestoorde microcirculatie en het bestuderen of acetylcarnitine of propionylcarnitine de werkzaamheid van carnitine overtreffen. Verder zou het zinvol zijn wanneer nieuwe analgetica worden ontwikkeld ter bestrijding van de neuropatische pijn bij CRPS (Hoofdstuk 13).

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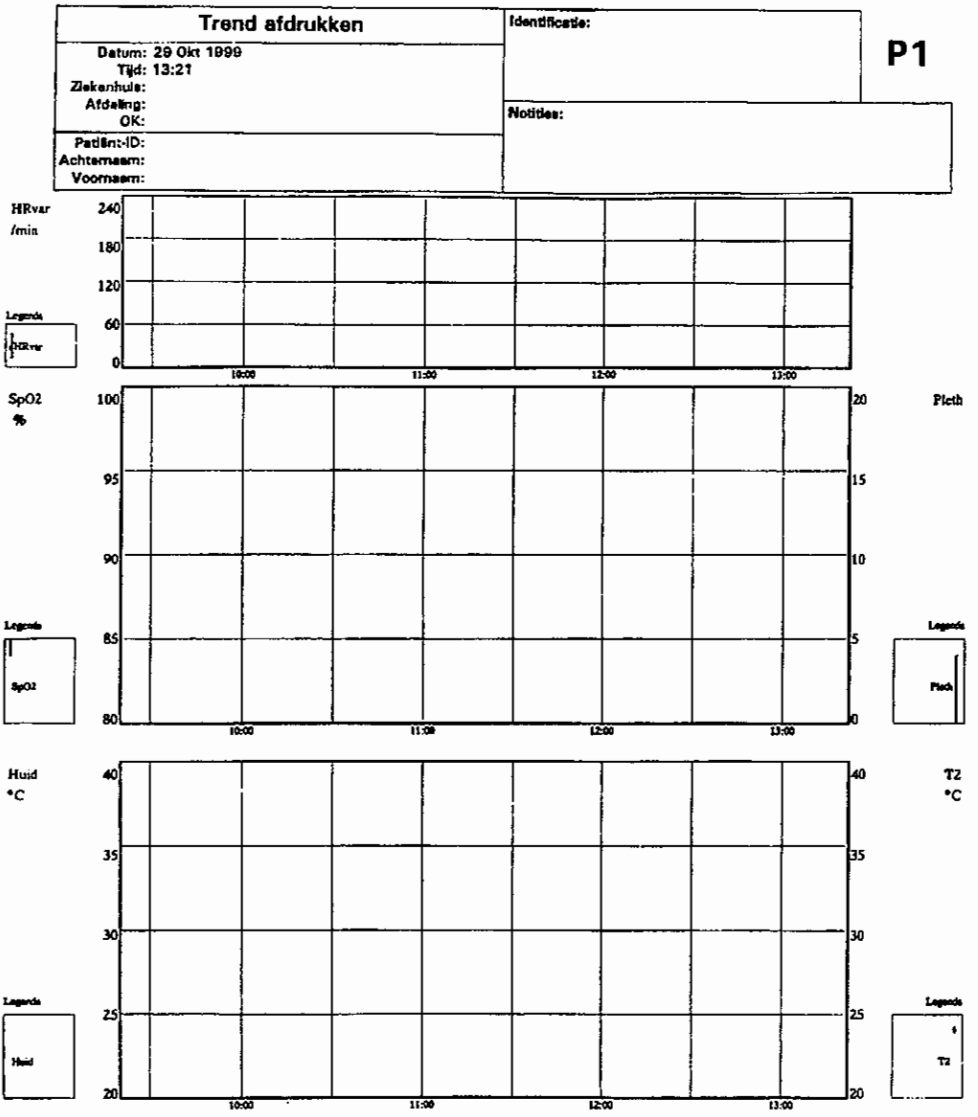
LIST OF ABBREVIATIONS

AC.....	acyl-L-carnitine
AVA.....	arteriovenous anastomosis
AVT.....	arterial vasoconstrictor tone
C2-C.....	acetyl-L-carnitine
C3-C.....	propionyl-L-carnitine
CRPS.....	Complex Regional Pain Syndrome
DMSO.....	dimethyl sulfoxide
EAA.....	excitory amino acids
EDRF.....	endothelium-dependent relaxation factor
FBF.....	forearm blood flow
FC.....	free L-carnitine
FiBF.....	finger blood flow
IL.....	interleukin
IASP.....	International Association for the Study of Pain
LCAC.....	Long-chain acylcarnitine
LPS.....	lipopolysaccharide
NAME.....	nitro-arginine methyl ester
NGF.....	nerve growth factor
NK.....	neurokinines
NMDA.....	N-methyl-D-aspartic acid
PKC.....	protein kinase C
RIS block....	regional intravenous sympathetic block
ROM.....	range of motion
RSDS.....	Reflex Sympathetic Dystrophy Syndrome
RSI.....	repetitive strain injury
SP.....	substance P
SpO_2	oxygen saturation measured by pulse oximetry
TC.....	total L-carnitine
TENS.....	transcutaneous electrical nerve stimulation
TNF.....	tumor necrosis factor
VAS.....	visual analogue scale
VIP.....	vaso-active intestinal polypeptide
VVT.....	venous vasoconstrictor tone
% FC.....	percentage FC of TC
FC(%).....	FC change in %, before ketanserin 100%
ΔTC(%).....	TC change in %, before ketanserin 100%

Appendix A: Registration form of CRPS treatment



Appendix B: Evaluation form of CRPS patients

Name: Gender: Birth date: Research nr:
Arm, hand/leg, foot:
Etiology:
Duration of complaints:
Previous therapy:
Plethysmographic changes after i.v. ketanserin
Skin temperature change after i.v. ketanserin
Pulse oximetric changes after i.v.ketanserin
Plethysmographic changes after i.v.carnitine
Skin temperature changes after i.v. carnitine
Pulse oximetric changes after i.v. carnitine.
Pain focus after ketanserin/carnitine treatment

	Start	3 months	6 months	9 months	12 months	15 months	18 months	21 months
Pain at rest								
Pain during exercise								
Impaired mobility								
Skin temperature								
Hyperhidrosis								
Edema								
Trophic changes								
Motor disturbances								
Hyperpathia/allodynia								

0 = absent, + = mild, ++ = moderate, +++ = severe

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CURRICULUM VITAE

Albert Moesker was born on August 11, 1947 in Winschoten, the Netherlands. He studied medicine at the University of Groningen and completed his specialist training in anaesthesiology at the University Hospital Groningen (Prof. Dr. J.C. Dorlas, Prof. Dr. D. Langrehr).

In the spring of 1980 he founded the Pain Management Department of the Refaja Hospital in Stadskanaal. Over the years the department has grown to a full-time pain management service available seven days a week. There is special focus on low back pain, cancer pain and the complex regional pain syndrome (CRPS). Regarding CRPS, he has presented many lectures at international congresses and published several papers on this topic.

These lectures and papers are the result of a continuous process of evaluation of treatment results and research. In this work support, in the past, was always given by Prof. Dr. W. Erdmann from the Department of Anesthesiology, Erasmus University Rotterdam. For final coordination and evaluation of biochemical determinations great help was given by Prof. Dr. H. R. Scholte from the Department of Biochemistry, Erasmus University Rotterdam. Plasma determinations were done at the Academic Medical Centre, University of Amsterdam Dept. Clinical Chemistry and Division Emma Children's Hospital, Laboratory Genetic Metabolic Diseases by colleague Dr. P. Vreken and Prof. Dr. R. J. A. Wanders. Exchange of knowledge on the metabolism of carnitine was conducted with emeritus Prof. Dr. W. C. Hülsmann of the Erasmus University Rotterdam and Prof. Dr. M. Calvani head of the research department of Sigma Tau, Pomezia, Italy.

From 1981 to 1998 the author was also medical director of the Refaja Hospital. He is a member of the pain chapter of the Dutch Society of Anaesthesiology, the Dutch chapter of the IASP, the World Association of Pain Clinicians, and the Dutch Society for the Study of Pain.

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