THE BIPHASIC BRADYKININ RESPONSE OF THE GUINEA-PIG ILEUM

PROEFSCHRIFT

ter verkrijging van de graad van
doctor in de geneeskunde
aan de Erasmus Universiteit te Rotterdam,
op gezag van de Rector Magnificus
Prof. Dr. C.J. van der Weijden
en volgens besluit van het college van dekanen.
De openbare verdediging zal plaats vinden op
Woensdag 17 October 1973
des namiddags te 3.00 uur

door

DAVID WILLIAM REID HALL

geboren te Woodford Green, England.

Promotor	:	Prof. Dr. I.L. Bonta
Co-referenten:		Dr. P.N. Aarsen
		Dr. P.R. Saxena

THE BIPHASIC BRADYKININ RESPONSE OF THE GUINEA-PIG ILEUM

A THESIS

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF MEDICINE

at the Erasmus University of Rotterdam
under the authority of the Rector Magnificus
Prof. Dr. C.J. van der Weijden
and according to the decision of the college of deans.
The public defence will take place on
Wednesday 17th October
at 3 p.m.

by

DAVID WILLIAM REID HALL

Supervisor : Prof. Dr. I.L. Bonta, Erasmus University Rotterdam.
Co-referees: Dr. P.N. Aarsen, University of Amsterdam.
Dr. P.R. Saxena, Erasmus University Rotterdam.
Ushers : Dr. B.A. Cooke.
Drs. A.L. Frankhuijzen.

Acknowledgements

I am indebted to Professor Bonta for so much constructive advice and continual encouragement throughout this research. For all the stimulating discussion with the staff of the Department of Pharmacology I am most grateful. Without this many of the ideas developed in this thesis would never have come to fruition. I should like to thank Dr. Aarsen and Dr. Saxena for their critical opinions of the manuscript and becoming co-referees of the thesis. In addition I should like to thank Mr. E.R. Verhoef for his competent assistance during part of this work, while he was engaged on his elective research program for third year medical student. I should also like to thank my ushers for undertaking their tasks.

Additionally I should like to express my thanks to Mr. J. van Dijk of the Audio-Visual Services Department for his excellent drawings, and to all those who have contributed to the production of the photographs. I am also in debt to all those that have contributed to the typing of the manuscript at all its various stages of preparation. In conclusion I should like to thank all the members of the Department of Pharmacology, both past and present, for making my stay at the Medical Faculty during the course of this work extremely pleasant and rewarding.

Cover photograph

Structural configuration suggested for bradykinin (Khairallah and Page, 1963, Ann. N.Y. Acad. Sci. *104*, 212.)

CONTENTS

CHAPTER 1 - LITERATURE REVIEW 9

HISTORICAL INTRODUCTION 9

THE KININ SYSTEM 11

THE ACTIONS OF KININS 14

INFLAMMATION 15

KININS AS MEDIATORS OF INFLAMMATORY RESPONSES 15

Do the actions of kinins resemble the features of inflammation? 15

Are kinins released at the site of injury? 16

Do conditions at the site of injury favour kinins? 17

SUGGESTED MECHANISMS OF ACTION OF BRADYKININ 17

The involvement of catecholamines. 18

The involvement of prostaglandins. 19

THE ACTIONS OF BRADYKININ ON SMOOTH MUSCLE 22

Antagonism 24

Potentiation 25

SOME IDEAS AND OBSERVATIONS 26

REFERENCES 29

CHAPTER 2 - THE BIPHASIC RESPONSE OF THE ISOLATED GUINEA-PIG ILEUM BY BRADYKININ 42

SUMMARY 42

INTRODUCTION 42

MATERIALS AND METHODS 44

RESULTS 45

Dose-response relationships 45

Specificity of the bradykinin relaxation 46

Conditions for the bradykinin relaxation 48

Variation in acetylcholine concentration 50

Action of bradykinin on its own response 53

DISCUSSION 53

REFERENCES 57

CHAPTER 3 - EFFECTS OF ADRENERGIC BLOCKERS ON THE RELAXATION OF THE GUINEA-PIG ILEUM BY BRADYKININ AND ADRENALINE 59

SUMMARY 59

INTRODUCTION 59
MATERIALS AND METHODS 60
RESULTS 61
α-Adrenergic antagonists 61
β-Adrenergic antagonists 63
α- and β-adrenergic blockers on the acetylcholine contraction 65
Combination of α- and β-adrenergic blockers 66
Action of phentolamine on the rat duodenum 67
DISCUSSION 68
REFERENCES 71

CHAPTER 4 - MECHANISM OF THE INHIBITORY ACTIONS OF SOME SPASMOGENS ON THE BRADYKININ RELAXATION OF THE GUINEA-PIG ILEUM 73
SUMMARY 73
INTRODUCTION 73
MATERIALS AND METHODS 74
RESULTS 75
Various spasmogens on the bradykinin relaxation 75
Low doses of angiotensin on the bradykinin relaxation 78
Morphine on the bradykinin relaxation 79
Atropine on the bradykinin relaxation 80
Morphine and atropine on the bradykinin reduction of its own response 81
DISCUSSION 82
REFERENCES 86

CHAPTER 5 - EFFECT OF PHENTOLAMINE ON THE BRADYKININ RELAXATION OF THE GUINEA-PIG ILEUM CONTRACTED BY VARIOUS SPASMOGENS 87
SUMMARY 87
INTRODUCTION 87
MATERIALS AND METHODS 87
RESULTS 88
Phentolamine reduction of the bradykinin relaxation after various spasmogens 88
Phentolamine effects on the contraction of the ileum by various spasmogens 90
DISCUSSION 92

CHAPTER 6 - EFFECTS OF SOME ANTI-INFLAMMATORY DRUGS ON THE BRADYKININ RELAXATION OF THE GUINEA-PIG ILEUM 94

SUMMARY 94

INTRODUCTION 94

MATERIALS AND METHODS 96

RESULTS 96

Anti-inflammatory drugs on the bradykinin relaxation 96

DISCUSSION 98

REFERENCES 100

CHAPTER 7 - POTENTIATION OF THE BIPHASIC BRADYKININ RESPONSE OF THE GUINEA-PIG ILEUM 102

SUMMARY 102

INTRODUCTION 102

MATERIALS AND METHODS 103

RESULTS 104

Cysteine and the bradykinin response 104

2,3-Dimercaptopropanol (BAL) and the bradykinin response 106

Synthetic bradykinin potentiating factor (BPP_{5a}) and the bradykinin response 107

Various potentiating agents and the acetylcholine contraction 109

DISCUSSION 110

REFERENCES 113

SUMMARY 115

SAMENVATTING 119

CURRICULUM VITAE 123

POST SCRIPT 124

Chapter 1

HISTORICAL INTRODUCTION

As early as 1909 hypotensive substances were found to be present in human urine. Indeed substances from human urine with hypotensive activity have been reported several times (Erdös, 1966), though extensive work to characterize the biochemical agents was not carried out until 1926 (Frey et al., 1950). The hypotensive activity was ascribed to a substance they termed kallikrein; the word derived from the Greek *kallikreas* for pancreas, since this organ was found to possess large amounts of the hypotensive activity. In 1937 it was demonstrated that a mixture of human plasma and an extract of salivary gland caused contraction of the isolated guinea-pig ileum (Werle, 1937). This substance was initially called Darmkontrahierende Substanz (Dk). It was further demonstrated to be a polypeptide originating from enzymatic cleavage of an α-globulin in plasma by kallikrein (Frey et al., 1950). The α-globulin was called kallidinogen and the polypeptide, kallidin.

In 1949 Rocha e Silva and his co-workers also described a hypotensive polypeptide in their studies on the effect of the venom of the snake *Bothrops jararaca* and trypsin on dog blood. They reported that incubation of the venom or trypsin with the pseudoglobulin fraction of plasma resulted in the formation of a potent vasodilator and smooth muscle stimulating substance. The experiments further indicated that these pharmacological actions were due to a substance whose destruction by enzymes in plasma or by a chemical means was consistent with the hypothesis that it was a polypeptide. When compared to histamine or acetylcholine the substance contracted the guinea-pig ileum slowly, and neither atropine nor anti-histamine agents antagonized its action. The polypeptide was called bradykinin to describe the slow contracting action. During purification of bradykinin both the hypotensive and spasmogenic actions remained inseparable (Rocha e Silva, 1960), indicating the presence of a single substance. At the time it was not possible to decide if bradykinin was similar to other substances formed from plasma, now known as plasma kinins.

In the following years the amino-acid composition and synthesis was worked out (Elliot et al., 1960b; Boissonnas et al., 1960). Initially it was indicated that bradykinin was an octapeptide (Elliot et al., 1960b), however synthesis of several octapeptides with the required structure only gave inactive peptides (Boissonnas et al., 1960). One of the synthetic peptides had nine amino-acids and had an activity similar to bradykinin (Elliot et al., 1960c). Re-examination of the original experimental data (Elliot et al., 1960c) showed that indeed bradykinin was a nonapeptide.

It was suggested from time to time that because of the similar properties of bradykinin and kallidin, that actually they were the same peptide. The relationship between kallidin and bradykinin was finally resolved when it was shown that human urinary kallikrein releases two polypeptides from human plasma (Pierce and Webster, 1961), one of these being a decapeptide, which they called kallidin-10, and the other a nonapeptide, kallidin-9. Kallidin-9 was found to be identical to bradykinin, while kallidin-10 had an extra lysine residue at the N-terminal carbon atom. At the same time Werle et al. (1961) independently came to the same conclusion.

Numerous additional kinins have now been found, the generic term 'kinin' being coined to apply to those polypeptides with properties similar to bradykinin and kallidin (Schachter, 1968). Some of these kinins include methionyl-lysyl-bradykinin, colostrokinin, neurokinin, wasp venom kinin and the kinins released by "permeability globulin". The structures of some kinins are shown in fig. 1. Not all the kinins have been fully characterized, but it is probable they will closely resemble bradykinin and kallidin.

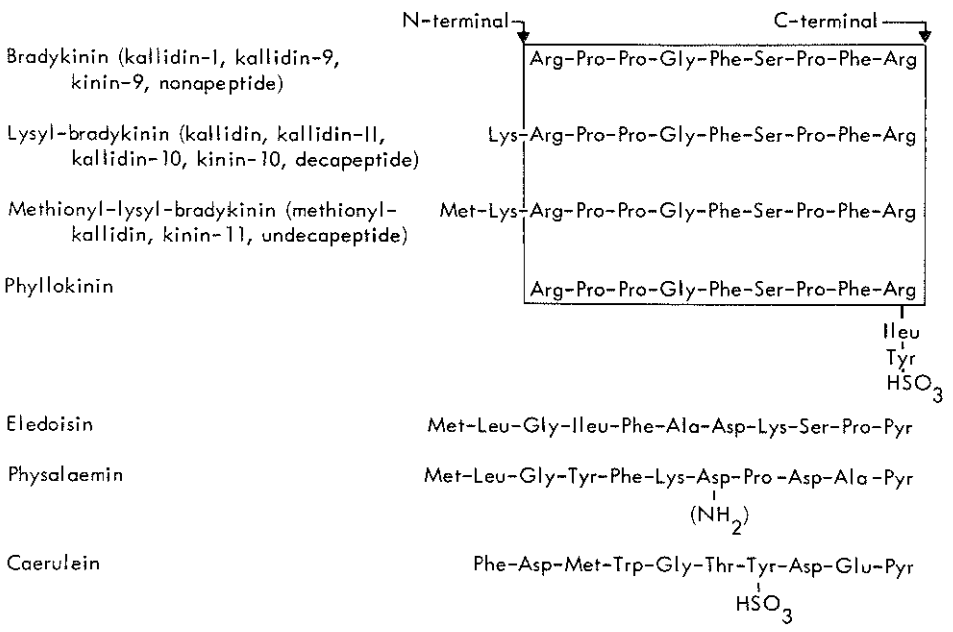

Fig. 1. Schematic repesentation of the chemical composition of some kinins and kinin-like peptides. The names in parentheses are synonyms. The basic kinin structure is indicated by the box.

THE KININ SYSTEM

In connection with the kinins two further factors should be considered, namely the production and destruction. On consideration of the production of kinins there are a number of substances that have assumed an important role, these being the "permeability factor/dil" (PF/dil) and the Hageman factor. PF/dil is a plasma protein that induces increased vascular permeability at the site of *intra dermal* injection, and was originally described as a factor present in the diluted serum of guinea-pigs (Mackay et al., 1953). The action of PF/dil was not blocked by anti-histamines, but was blocked by agents that block kallikrein. At the time it was not clear whether the two agents were really different, though it was later shown that PF/dil and Hageman factor could be separated chromatographically (Becker and Kagen, 1964). It is now considered that PF/dil activates kalli-

krein, which in turn results in the release of kinins (Mason and Miles, 1962).

Hageman factor was originally described as a clotting factor congenitally missing from the plasma of Mr. John Hageman (Ratnoff, 1966). The Hageman factor is a plasma protein, that after activation by glass or other particulate surfaces can initiate a series of reactions, called the *Intrinsic clotting mechanism*. PF/dil does not form in blood protected from contact with glass. It was suggested that Hageman factor might be an essential step in the activation of PF/dil (Margolis, 1959). It has been postulated that human plasma contains two kinin-forming systems (Vogt, 1966; Vogt et al., 1967) (fig. 2a), in which kininogenase II is activated directly by Hageman factor, but kininogenase I (kallikreinogen) indirectly via PF/dil. They also concluded that kininogenase II activates kininogenase I. Kininogenase I has been equated to serum kallikrein (Vogt, 1966). Kininogenase I is identical, functionally, with the bovine kininogen preparation of Habermann et al. (1963).

The type of kinin produced is dependent on the nature of the substrate and the kallikrein. It is known that pig serum kallikrein produces bradykinin from bovine kininogen, whereas pancreatic kallikrein produces kallidin (Habermann, 1966). When meth-lys-bradykinin is the substrate serum kallikrein forms bradykinin, but the pancreatic kallikrein is inactive. These results indicated that plasma kallikrein is not formed in the pancreas. Kallidin, formed by the action of kallikrein, can be converted into bradykinin by an aminopeptidase present in blood plasma (Webster and Pierce, 1963; Erdös et al., 1963). Trypsin has also been found to be active in converting kallidin to bradykinin (Webster and Pierce, 1963) and furthermore has been shown to liberate a bradykinin-like substance from kininogen, possibly both directly and indirectly by activating plasma kallikrein (Werle, 1963; Ferreira and Rocha e Silva, 1969).

Kinins are rapidly destroyed in plasma and most other body fluids, the enzymes that destroy the kinins being collectively known as kininases. In human plasma the enzyme is called carboxypeptidase-N, and has been shown to break the Phe-Arg bond in bradykinin or kallidin (Erdös and Sloane, 1962). Recently it has been shown that the lung kininases are more important for the removal of circulating kinins

than the plasma kininases (Ferreira and Vane, 1967; Alabaster and Bakhle, 1972). The kininases in plasma have been shown to have an enhanced activity in the presence of adrenaline (Mashford and Zacest, 1967), suggesting a possible control mechanism.
Further discussion of the kininases will be given in a later section.

These observations have lead to the proposal of the reaction sequence (fig. 2a,b) in human plasma resulting in the formation (Miles, 1964; Becker and Kagen, 1964) and destruction (Erdös, 1966) of kinins. Complete biochemical proof is still unavailable, but the existing evidence supports such a pathway.

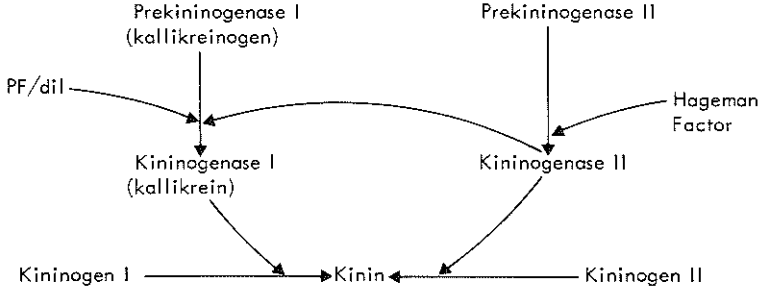

Fig. 2a. Schematic representation of the two kinin-forming systems present in human plasma (Vogt, 1966). Kininogenase I is the enzyme absent from glass treated plasma; kininogenase II is the enzyme present in active form after glass contact. Kininogen I is the substrate largely left intact; kininogen II is the substrate consumed during glass activation of plasma.

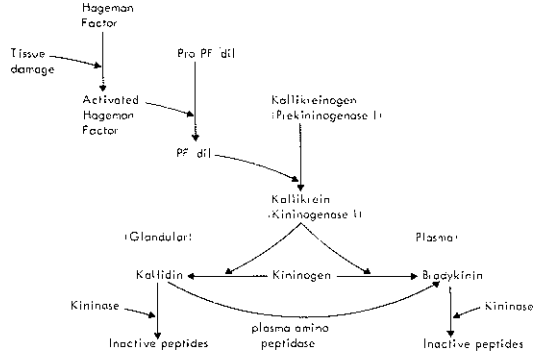

Fig. 2b. Schematic representation of one of the biochemical pathways for the formation and destruction of kinins. In some cases kallikrein produces kallidin (see text), which can be converted into bradykinin by an aminopeptidase.

THE ACTIONS OF KININS

Kinins have been implicated in several physiological processes, including regulation of blood flow in the salivary gland (Hilton and Lewis, 1956), constriction of the ductus arteriosus and conversion of the foetal blood circulation to that of an adult (Melmon et al., 1968). They have also been shown to be capable of contracting smooth muscle (Rocha e Silva et al., 1949), inducing hypotension (Rocha e Silva et al., 1949), increasing blood flow (Hilton and Lewis, 1956) and microvascular permeability (Holdstock et al., 1957), inciting pain (Armstrong et al., 1957) and possibly causing the migration of granulocytic leukocytes (Graham et al., 1965; Spector and Willoughby, 1964).

It has been proposed that kinins have a primary etiological role in the development of the acute inflammatory response, and as such are thought to be implicated in the pathogenesis of various forms of acute arthritis (Melmon et al., 1967; Kellermeyer, 1967), asthma (Herxheimer and Stresemann, 1961; Abe et al., 1967), foreign body granuloma and pneumoconiosis (Warren and Kellermeyer, 1968), endotoxin shock (Nies et al., 1968), the Schwartzman reaction (Halpern, 1964), heriditary angioneurotic oedema (Donaldson, 1968), migraine headaches (Ostfeld et al., 1957) and acute inflammation associated with thermal burns (Rocha e Silva and Rosenthal, 1961).

The following sections of the introduction will be devoted to giving the reader an insight into the evidence for proposing kinins as mediators of some acute inflammatory reactions, and also some current ideas concerning the mechanisms. In these sections will be discussed the involvement of catecholamines and prostaglandins. These ideas will be extended to the actions of bradykinin on vascular and non-vascular smooth muscle, together with the known antagonists and potentiators of bradykinin. In the final section of the introduction will be presented some of the initial ideas and observations that have directed the work of this thesis.

INFLAMMATION

The reaction of a tissue to sub-lethal injury is known as inflammation. In its acute form it is characterized by vasodilatation, increased vascular permeability and oedema, pain and the migration and extravascular accumulation of leukocytes. With severe injury these events occur almost simultaneously, however with milder stimulus there is a characteristic order of responses. The increase in the vascular permeability to a mild stimulus tends to be biphasic, with an earlier, transient phase and a delayed, sustained phase (Spector, 1964; Wilhelm and Mason, 1960).

In models of mild inflammation the vascular permeability present in the early transient phase has been shown by the colloidal labelling techniques to involve only the small venules (Wells and Miles, 1963), while during the delayed phase the capillaries also become heavily labelled (Wells and Miles, 1963; Cotran and Majno, 1964; Spector et al., 1965) depending on the intensity and nature of the stimulus. The results with capillaries do not necessarily indicate that there is a great exudation of fluid or protein, since electron microscopy has revealed that carbon can be trapped in fibrin plugs within the vessel (Cotran, 1965).

KININS AS MEDIATORS OF INFLAMMATORY RESPONSES

Do the actions of kinins resemble the features of inflammation

As described earlier bradykinin can cause vasodilatation, increased vascular permeability and oedema, and pain (Elliot et al., 1960a; Konzett and Stürmer, 1960; Lewis, 1970). Kinins appear to be relatively ineffective at promoting migration of leukocytes (Spector and Willoughby, 1964). At high concentrations Lewis (1962) observed migration and also sticking of leukocytes to the venules of the mesentary, but this was not confirmed by Zweifach (1966). Nevertheless sticking has been observed for the rabbit ear chamber (Graham et al., 1965). Convincing evidence as to the precise role of kinins in migration and sticking of leukocytes is unavailable.

Are kinins released at the site of injury

The research investigator is presented with a large array of difficulties in the study of kinins, since they are so rapidly destroyed in the body by kininases (Allwood and Lewis, 1964; Ferreira and Vane, 1967), and thus are extremely difficult to measure accurately in inflammatory exudates. Nevertheless there are a number of reports of kinins being found with other mediators at the site of local injury. One of these experimental injuries is moderate to severe treatment by heat. Histamine and a bradykinin-like substance can be shown to be released after scalding the skin of rats and collecting the fluid in an air pouch submitted to a temperature of 96°C for 15 sec (Rocha e Silva and Rosenthal,1961). Under less drastic conditions, 54°C, histamine is still released together with the production of a bradykinin-like material (Wilhelm and Mason, 1960); though under milder conditions, 43-46°C, histamine is no longer released (Rocha e Silva and Antonio, 1960; Starr and West, 1967). At the latter temperature it is known that mast cells are stabilized (Mongar and Schild, 1957). Correlation was found to exist between the anti-bradykinin activity of some substances *in vitro* and the oedema reducing properties (Garcia Leme and Rocha e Silva, 1965). It can also be shown that substances interfering with the release of bradykinin from its active precursor in plasma, such as hexadimethrine, inhibited the development of the heat oedema (Garcia Leme et al., 1967, 1970) and the yeast oedema (Kellett, 1965). Sulphated polysaccharides, which are known to deplete the labile pool of bradykininogen, reduced the heat oedema (Garcia Leme et al., 1967). More recently it has been suggested that the subcutaneous tissue elicits a process leading to plasma extravasation and that subcutaneous tissue is the chief site of release of the bradykinin-like material (Garcia Leme et al., 1970).

In addition to the thermic oedema, kinin release has been demonstrated in the carrageenin oedema (Di Rosa and Sorrentino, 1968) and the kaolin oedema (Bonta and de Vos, 1966, 1967). Further evidence for kinin involvement came from experiments of Edery and Lewis (1963), who although could not detect an increase in kinin in the lymph draining an injured hind leg of the dog, did, however, find an in-

crease in kinin-forming activity.

Do conditions at the site of injury favour kinins

The formation and accumulation of plasma kinins may be dependent on the conditions existing in inflammed tissues. It has been considered that proteolytic enzymes are activated in tissue injury (Beloff and Peters, 1945; Ungar, 1947), although it is unclear exactly what factors are involved. The acidic environment that prevails in tissue damage is thought to activate kallikrein from its inactive precursor (Werle, 1934). Further was suggested that in conditions where acid metabolites are formed, kallikrein is responsible for the vascular effects (Frey et al., 1950). It is still not clear whether changes of pH in damaged tissue are sufficient to cause activation.

In addition to bradykinin it has been shown that the kinin, methionyl-lysyl-bradykinin can be formed after acid activation of kininogenases in plasma (Edery and Lewis, 1962). The latter peptide had a more prolonged effect than bradykinin, which is considered to be attributable to its possible greater resistance to destruction by kininases, or conversion to kallidin and bradykinin. Kininases themselves appear to be strongly dependent on pH (Edery and Lewis, 1962). These authors showed that phosphate buffer, pH 6.0, effectively inhibits kininase. At this pH it has been thought that kinin formation is decreased (Trautschold, 1968), though incompatible evidence exists (Edery and Lewis, 1962; Zachariae et al., 1967). Further work by Aarsen and Kemp (1962) showed that cysteine hydrochloride solutions (often used to potentiate bradykinin's smooth muscle effects - see later section) needed to be neutralized to exclude pH effects on the kininases. It seems thus possible that an acid environment provides not only the suitable conditions for the formation and accumulation of kinins, but may even result in the formation of more stable kinins.

SUGGESTED MECHANISMS OF ACTION OF BRADYKININ

Of the kinins investigated it appears that bradykinin is the most important and the most potent mediator of vascular permeability

(Elliot et al., 1960a; Collier, 1961; Frimmer, 1960; Stürmer and Cerletti, 1961; Wilhelm, 1962). The possibility has been advanced that catecholamines play a fundamental role in the mechanism of action of bradykinin (Rocha e Silva, 1964). Two schools of thought have predominated, one suggesting that catecholamines have a pro-inflammatory effect, while the other an anti-inflammatory effect.

The involvement of catecholamines

Bradykinin has been shown to liberate catecholamines from the adrenal medulla (Lecompte et al., 1961; Feldberg and Lewis, 1964) and also to stimulate the sympathetic ganglia (Lewis and Reit, 1965). In the latter respects bradykinin resembles non-nicotinic ganglionic stimulants (Lewis and Reit, 1966; Trendelenburg, 1966). Further it has been shown that adrenergic blockade and adrenalectomy potentiate the hypotensive (Rocha e Silva et al., 1960; Lloyd, 1962; Nakano, 1965) and bronchoconstrictor actions of bradykinin (James, 1969). There is also evidence available indicating that adrenaline is able to activate proteases *in vivo* (Biggs et al., 1947; Macfarlane and Biggs, 1948). This could start a process leading to the release of bradykinin or related kinins. Bradykinin has been shown to be released from the lungs of rabbits on treatment with doses of catecholamines that induce pulmonary oedema (Di Mattei, 1962). Also it has been shown that the kaolin-induced oedema of the rat paw, where there is considered to be kinin release, can be suppressed by guanethidine and reserpine treatment (Bonta and De Vos, 1966), further suggesting a pro-inflammatory effect of catecholamines.

Contrary to these ideas it has been proposed that catecholamines generally have an anti-inflammatory effect. Inhibitors of the enzyme monoamine oxidase can suppress acute inflammatory responses (Northover, 1963) and also inhibitors of the enzymes DOPA-decarboxylase and dopamine-β-hydroxylase leads to a significant reduction of deranged permeability (Spector and Willoughby, 1960; Willoughby and Spector, 1964). These results suggest that the development of the vascular reaction to injury depends partly on the inactivation not only of adrenaline, but also of noradrenaline, DOPA and dopamine, and that undestroyed these compounds might act as local anti-inflammatory agents. Other indirect

evidence supporting the latter ideas has been presented by Möller (1962), who showed the disappearance of catecholamines from recently injured tissue, Raekalli (1963), who demonstrated an increase in monoamine oxidase activity in damaged skin, Jacques (1965), who found suppression of the anti-inflammatory activity of amidopyrine by α-methyl-p-tyrosine, and Gözsy and Kátó (1966), who found a delayed sensitization to histamine by catecholamines. More direct evidence was presented by Northover and Subramanian (1962), who showed that noradrenaline and phenylephrine (*s.c.*) reduced the formaldehyde induced oedema of the mouse paw, and Brown and West (1965), who demonstrated that adrenaline (*i.d. and s.c.*) blocked the dextran and egg white oedema. More recently catecholamines have been confirmed to have an anti-inflammatory action on the carrageenin-induced rat paw oedema, though not in bilaterally adrenalectomized animals (Bhalla et al., 1970). This suggested a mechanism related to the pituitary-adrenal system, and not to local vasoconstriction as suggested by Willoughby and Spector (1964).

The experiments of Brown and West (1965) showed that not only did adrenaline block the dextran and egg white oedema, but also exogenously administered glucose. Adrenaline was much more potent than glucose. A remarkable correlation has been reported between the anti-oedemous activity of adrenaline and its hyperglycaemic effects (Kellett, 1965), in particular was shown that glucose inhibited the oedema, while insulin antagonized both the adrenaline and glucose reduction of the oedema. It would seem that the anti-inflammatory properties of adrenaline are mediated solely through its hyperglycaemic effects. It is apparent from the evidence presented that where catecholamines are released there is a greater likelyhood of an anti-inflammatory than a pro-inflammatory effect, and that this effect may be mediated by an action on glucose mobilization. Nevertheless it seems unlikely that the increase in vascular permeability and the development of the inflammatory response to bradykinin is mediated via a release of catecholamines.

The involvement of prostaglandins

The plasma kinins and the prostaglandins are alike in two important

ways. Firstly, both are endogenous substances that can be liberated locally in different parts of the body, and can be rapidly destroyed. Secondly, both kinins and prostaglandins potently affect smooth muscle, blood vessels and nerves. The prostaglandins are structurally unrelated to the kinins. The chemical relationships between some of the important prostaglandins are shown in fig. 3

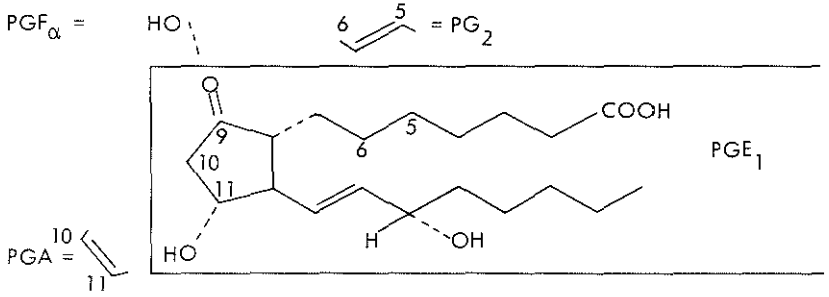

Fig. 3. The relationship of prostaglandin E_1 (PGE_1) to other prostaglandins. PG_2s have additionally a 5,6-trans double bond; PGFs have an α- or β-hydroxy instead of oxo at carbon 9; PGAs are dehydrated analogues of PGEs with a double bond between carbon 10 and 11 in the ring. PGBs are not shown, but are isomers of PGAs with a double bond between carbon 8 and 12, instead of 10 and 11.

Unlike kinins, not all prostaglandins have similar actions (Horton, 1969). Prostaglandin E_1 resembles bradykinin in contracting the rat uterus and the guinea-pig ileum, and relaxing the rat duodenum, but differed from bradykinin in not relaxing the acetylcholine contracted guinea-pig ileum (Hall, unpublished observation). Both bradykinin and PGE_1 dilate the blood vessels of the rat hind limb, and increase the permeability of the capillaries of the guinea-pig skin (Horton, 1963). An E-type prostaglandin has been found in the exudate during the secondary phase of the carrageenin oedema in the rat (Willis, 1969), and also it is known that *i.d.* injections of PGE_1 or PGE_2 in rat or man produce inflammatory responses (Crunkhorn and Willis, 1971). Bradykinin and $PGF_{2\alpha}$ both produce bronchoconstriction of the guinea-pig lungs (Collier and Shortley, 1960; Berry and Collier, 1964). Antagonism of the bronchoconstriction induced by bradykinin has been demonstrated both *in vitro* and *in vivo* (Graeff and Moog,

1964; Collier and Shortley, 1963), also antagonism of the $PGF_{2\alpha}$ contractions of the human isolated bronchial muscle (though not of the PGE_1 and PGE_2 relaxations) has been demonstrated by the aspirin-like drugs (Collier and Sweatman, 1968). These results together with the similarities between the actions of kinins and prostaglandins raised the question as to whether the non-steroidal anti-inflammatory drugs have their action by antagonizing the effects of $PGF_{2\alpha}$ or an allied prostaglandin (Collier and Sweatman, 1968). During anaphylaxis of the guinea-pig lungs it was found that histamine, slow reacting substance in anaphylaxis (SRS-A), rabbit aorta contracting substance (RCS) and prostaglandins E_2 and $F_{2\alpha}$ were released (Piper and Vane, 1969). They also found that the release of RCS could be antagonized by the aspirin-like drugs. These same anti-inflammatory agents were found to interfere with the action of SRS-A (Berry and Collier, 1964) released during challenge of the isolated sensitized guinea-pig lungs, also arachidonic acid (Berry, 1966) and slow reacting substance-C (SRS-C), produced when egg yolk lecithin or guinea-pig lung is challenged by venoms containing phospholipase-A (Vargaftig et al., 1969). With the finding that arachidonic acid releases RCS from the isolated lungs, and that this release is also antagonized by the aspirin-like drugs (Vargaftig and Dao, 1971), it was suggested that RCS may be an intermediate in the synthesis of prostaglandins from arachidonic acid. It has now been demonstrated that synthesis of prostaglandins by homogenates of guinea-pig lung can be inhibited by the aspirin-like drugs (Vane, 1971); also, the adrenaline induced synthesis of prostaglandins in the isolated dog spleen (Ferreira et al., 1971); and the production of prostaglandins induced by thrombin in human platelets (Smith and Willis, 1971). The evidence points to the possibility of bradykinin being able to induce a release (*de novo* synthesis) of prostaglandin-like material from arachidonic acid or other precursors (Berry and Collier, 1964; Berry, 1966; Piper and Vane, 1969). The fact that the antagonistic activity of aspirin on prostaglandin synthesis parallels the anti-inflammatory activity in many situations argues strongly for a central position of prostaglandins in the pathogenesis of inflammation. The direct activity of aspirin on the $PGF_{2\alpha}$ contraction of isolated human bronchial muscle, appears to be contradictory to the above hypothesis, however Collier

(1971) believes this action to be a preferential reinforcement of antagonism against $PGF_{2\alpha}$ distinct from PGE_1 and PGE_2.

THE ACTIONS OF BRADYKININ ON SMOOTH MUSCLE

The subject matter has so far been limited to study of the various agents involved in the production of lesions on vascular and bronchial smooth muscle that give rise to the inflammatory response. To enable a fuller understanding an appraisal is also needed of the direct effects of bradykinin on vascular smooth muscle.

A variety of responses have been found in vascular smooth muscle towards kinins. Kinins constrict the sheep coronary arteries (Kovalčík, 1962) and ductus arteriosus (Kovalčík, 1963), canine femoral and mesenteric arteries (De Pasquale and Burch, 1968), bovine pulmonary arteries (Klupp and Konzett, 1965), and the rabbit aorta, either normal or reserpinized (*in vitro*) (Türker and Khairallah, 1966). In the case of the rabbit mesenteric vein strip, high concentrations of bradykinin contract but low concentrations transiently inhibit the spontaneous activity (Sutter, 1965; Somlyo and Somlyo, 1970). The canine large coronary arteries are also not contracted by bradykinin (Kovalčík, 1962), and moreover the total perfused coronary bed undergoes considerable vasodilatation (Antonio and Rocha e Silva, 1962). Of interest is the work of Somlyo and Somlyo (1970), who found that bradykinin relaxes small strips of rabbit mesenteric artery contracted by noradrenaline. The most characteristic response of bradykinin in mammalian cutaneous, muscular and splanchnic beds is vasodilatation. The vasodilator action of bradykinin is considered to be direct, occuring in denervated vascular beds (Fregnan and Glässer, 1968), after sympathetic blockade or atropine (Lloyd, 1962; Mason and Melmon, 1965; Rosas et al., 1965), β-adrenergic blockers (Chou et al., 1965; Skinner and Powell, 1967; Skinner and Webster, 1968) and reserpinization (Miele and De Natale, 1966). The cellular mechanisms of the vasodilator or constrictor actions of bradykinin, and a classification of the diverse actions is still unresolved.

Besides vascular smooth muscle other smooth muscle preparations can be contracted by kinins, and in particular bradykinin. These

include the rat uterus and stomach fundus strip, the rabbit duodenum and large intestine, and the guinea-pig ileum (Rocha e Silva, 1970; Trautschold, 1970). The mechanism of the contraction of the isolated rat uterus and the guinea-pig ileum to synthetic bradykinin is thought to represent both a direct action of the kinin on the smooth muscle (Day and Vane, 1963; Khairallah and Page, 1963; Wiegershausen et al., 1964; Gershon, 1967) and also indirectly via the release of acetylcholine (Wiegershausen et al., 1964). The former mechanism was proposed since no other indirect mechanism could be demonstrated, and further that tetrodotoxin, eserine and hyoscine did not affect the maximal response with bradykinin. The latter mechanism was the result of the finding that contraction of the isolated guinea-pig ileum could be inhibited by atropine and morphine, and potentiated by eserine. The inhibition was manifested by a reduction and the potentiation by an increase in the contraction speed of the organ.

Although bradykinin is known to contract many smooth muscle preparations, it is generally thought that, as exceptions, the rat duodenum (Elliot et al., 1960a) and the hen rectal caecum (Erdös, 1966) are relaxed. These effects have been considered to be one of the characteristic features of the pharmacology of bradykinin (Rocha e Silva, 1970). Indications are present in the literature that suggest bradykinin to be able to relax the rabbit duodenum (Fishlock, 1966), the guinea-pig taenia coli (Regoli and Vane, 1964) and the rat ileum (Sherman and Gautieri, 1969). A sympathomimetic mechanism has been proposed for the bradykinin relaxation of the rat duodenum (Türker et al., 1964; Montgomery, 1968), but evidence incompatible with this has been presented (Antonio, 1968). It has further been suggested that the bradykinin relaxation of the guinea-pig taenia coli is via an action on conducted impulses or spontaneous spike activity or both. This conclusion was derived from experiments using the sucrose gap technique, whereby bradykinin was shown to mainly suppress spontaneous spike activity, though did exhibit small hyperpolarizations (Aarsen and Van Caspel-de Bruyn, 1970).

ANTAGONISM AND POTENTIATION OF BRADYKININ RESPONSES

At this point in the introduction it would be instructive to discuss the current knowledge concerning antagonists of kinin responses on smooth muscle, and also the range of drugs that are known to potentiate kinins.

Antagonism

There are unfortunately no known specific antagonists to bradykinin, even though intensive research activity has been devoted to the finding of one. However, an accumulation of information concerning drugs with some activity against bradykinin can be profitably discussed. Contraction of the guinea-pig ileum by bradykinin has been shown to be resistant to atropine, anti-histamines and paralyzing concentrations of nicotine (Ambache and Rocha e Silva, 1951; Werle, 1955; Walaszek et al., 1963), and unaffected by morphine or BOL-148 (Walaszek et al., 1963). However, as previously described, Wiegershausen et al. (1964) found that contraction of the isolated guinea-pig ileum could be inhibited by atropine and morphine, and potentiated by eserine. The inhibitory phase of bradykinin on the rabbit intestine is blocked by morphine, but not by atropine, local anaesthetics, adrenergic blockers, guanethidine or phenylbutazone. In the rat intestine the inhibitory effect is not blocked by any of these agents or morphine (Bauer et al., 1966).

Of the numerous compounds examined it has been reported that some phenothiazines, imipramine and cyproheptidine can act as antagonists (Rocha e Silva and Garcia Leme, 1963, 1964, 1965; Garcia Leme and Rocha e Silva, 1965). Also they reported that some dibenzocycloheptene derivatives were competitive antagonists, while benzodiazepine, cycloalkindole and dibenzazepine derivatives acted as non-competitive antagonists. Methixine was believed to be a competitive antagonist, but Van Riezen (1966) reports a non-competitive antagonism. The latter studies were on the guinea-pig ileum.

Carboxypeptidase-B effectively antagonizes the actions of bradykinin *in vivo* (Erdös et al., 1963). However the rationale of its action is rapid destruction of the kinins, rather than a direct action

on a receptor. The antagonistic action of some anti-inflammatory drugs on the bradykinin bronchoconstriction of the guinea-pig lungs is of great importance, and has been discussed elsewhere in the introduction.

Potentiation

Many compounds are known to potentiate the actions of bradykinin. One such group of agents consists of the thiol compounds. These include cysteine, α-thiol glycerol, 2-mercaptoethanol, 2,3-dimercaptopropanol (BAL) and thioglycolic acid (Ferreira and Rocha e Silva, 1962; Picarelli et al., 1962; Erdös and Wohler, 1963a,b). All these compounds can inhibit the enzymatic breakdown of kinins, and it is thought that their *in vivo* effects are related to this (Erdös and Wohler, 1963a,b). With isolated smooth muscle preparations the mechanism is uncertain, there existing evidence that the effect of bradykinin is potentiated by a direct action on the muscle. Much evidence for a direct potentiating action of thiols on isolated smooth muscle preparations has been proposed (Cirstea, 1965; Doleschel and Auerswald, 1966; Auerswald and Doleschel, 1967; Tewksbury, 1967). More recently a cholinergic mechanism has been suggested (Potter and Walaszek, 1972). In the same way that thiol compounds potentiate the effects of bradykinin, a bradykinin potentiating factor (BPF) isolated from the venom of *Bothrops jararaca* (Ferreira and Rocha e Silva, 1962; Ferreira, 1965) has also been found to be active. BAL and BPF have recently been examined on the relaxation and contraction responses of the rat intestine by bradykinin (Camargo and Ferreira, 1971). No potentiation was found of the relaxation. The authors regard their results as an indication of separate receptors for contraction and relaxation.

Potentiation of the bradykinin contraction of the guinea-pig ileum can also be demonstrated with the local anaesthetic, procaine, though cocaine and tetracaine only sensitized the ileum to further doses of bradykinin (Wiegershausen et al., 1973). Various proteins e.g. chymotrypsin and trypsin are also able to potentiate the action of bradykinin on the guinea-pig ileum and the rat uterus (Edery, 1964, 1965). It seems to be quite specific for kinins, though the mechanism is unknown. In a recent paper the action of chymotrypsin was regarded to

be sensitization rather than potentiation (Wiegershausen et al., 1973). It is also known that the guinea-pig ileum can be sensitized to the action of bradykinin by trypsin hydrolysates of ox and rabbit plasma (Aarsen, 1968) and the rat uterus by peptide-B, produced by the action of thrombin and bovine fibrinogen (Gladner et al., 1963; Gladner, 1966). These peptides may be of importance in sensitizing tissue receptors to bradykinin.

SOME IDEAS AND OBSERVATIONS

The involvement of catecholamines in the inflammatory responses has been treated in earlier sections. The precise nature of the mechanism is unknown, and also if catecholamines are pro- or anti-inflammatory substances. It seemed that the mechanism of the possible interactions between bradykinin and catecholamines could be a fruitful area of research.

Evidence is available suggesting that catecholamines can assist or potentiate the activities of kinins (Rocha e Silva et al., 1960; Lloyd, 1962; Di Mattei, 1963; Nakano, 1965; Bonta and De Vos, 1966; James, 1969), though equally there is evidence suggesting catecholamines to have an anti-inflammatory (anti-bradykinin?) action (Spector and Willoughby, 1960; Northover, 1963; Willoughby and Spector, 1964; Brown and West, 1965; Northover and Subramamian, 1966). It has been further suggested that the anti-inflammatory activity of catecholamines is mediated entirely through their hyperglycaemic action (Kellett, 1965). It is now well established that the hyperglycaemic action of adrenaline is due to stimulation of the rate of formation of adenosine 3',5'-cyclic monophospate (cyclic AMP), which in turn increases the rate of formation of active phosphorylase (Robison et al., 1968).

There is an accumulation of evidence, albeit mostly indirect, that cyclic AMP could play a role in the inflammatory response. The β-adrenergic blocker, dichloroisoprenaline, was found to decrease the isoprenaline reduction of the formaldehyde oedema in the mouse paw, but not of the reduction due to noradrenaline and phenylephrine (Northover and Subramamian, 1962). It has also been shown that the adrenaline reduction of the dextran oedema in the rat paw is anta-

gonized by the β-adrenergic blocker, sotalol (McKinney and Lish, 1966), and that sotalol also blocked the catecholamine reduction of the formaldehyde oedema in rats (Brown et al., 1968). Catecholamine antagonism of the various oedemas appears to be via a stimulation of β-adrenergic receptors. There is strong evidence to suggest that the enzyme adenyl cyclase (the enzyme responsible for the formation of cyclic AMP from ATP) fits the definition of a β-adrenergic receptor. The studies that support this concept come from liver, heart, brain and avian erythrocytes (Robison et al., 1968). Studies on smooth muscle systems are, on the whole, compatible with the hypothesis that the relaxing effect of catecholamines is mediated by adrenergic receptors, and is related to increases in the levels of cyclic AMP. The hypothesis has been extended to non-adrenergic systems, since papavarine, a smooth muscle depressant, inhibited the action of phosphodiesterase (the enzyme that degrades cyclic AMP) thus allowing an increase in cyclic AMP (Triner et al., 1970; Kukovetz and Pöch, 1970). These results might well be extended to vascular smooth muscle. Direct evidence implicating cyclic AMP as a local anti-inflammatory hormone has come from experiments using theophylline (a phosphodiesterase inhibitor) and cyclic AMP, where both agents were shown to display clear cut dose-related anti-inflammatory activity on the carrageenin, dextran and egg white oedemas of the rat paw (Bertelli et al., 1966).

ATP (the precursor of cyclic AMP) has also been shown to be active in causing bronchoconstriction of the guinea-pig lungs (Collier and Shortley, 1963), and as with bradykinin and SRS-A the action is blocked by low doses of aspirin. Perfusion of the unsensitized guinea-pig lungs with ATP did not result in the release of RCS or prostaglandins, as is found with bradykinin and SRS-A, or in anaphylaxis (Piper and Vane, 1969). These results suggest that aspirin does not block the action of ATP by a mechanism involving the inhibition of the synthesis of prostaglandins (Vane, 1971). Aspirin may interfere directly with ATP, though it is possible that ATP must first be converted into an active principle (cyclic AMP?).

The results so far described prompted a consideration of the factors that may be interrelated between the catecholamines and bradykinin with particular reference to cyclic AMP. As a starting point

for experimentation it seemed more feasible to study a relatively simple system, as opposed to the complexities of the inflammatory syndrome. Of the systems available where it is known that bradykinin and catecholamines show activity, is smooth muscle, which is also desirable on account of its connection with inflammation. Another advantage of the use of smooth muscle preparations is that the various parameters can be rigidly controlled, allowing more precise and detailed investigations. Initially the rat uterus and the guinea-pig ileum were chosen, to examine the influence of the cyclic AMP system. Unfortunately no significant effects of increased cyclic AMP levels on the bradykinin responses were found. One of the experimental conditions that was employed required the depletion of tissue cyclic AMP by incubation with imidazole (Robison et al., 1968). Under this condition a sustained contraction of the guinea-pig ileum was observed. Subsequent addition of bradykinin to this organ, now with increased tone, resulted in the appearance of a small relaxation immediately followed by a contraction. This small bradykinin relaxation provoked considerable curiosity. Relaxation of the guinea-pig ileum does not seem to have been previously reported for bradykinin. As a first step in the characterization of this bradykinin relaxation response, the ileum was challenged by acetylcholine, which resulted in a maintained contraction. On further addition of bradykinin a biphasic response was seen, first a relaxation immediately followed by a contraction. An investigation into this hitherto unknown bradykinin relaxation on intestinal smooth muscle may lead to results applicable to vascular smooth muscle and allow the development of a model for some of the actions of bradykinin in inflammation.

The present investigations have examined the conditions for and characteristics of the bradykinin relaxation of the isolated guinea-pig ileum. The involvement of catecholamines and prostaglandins has also been examined, and further some potentiators of this previously unknown response.

REFERENCES

Aarsen, P.N., 1968, Sensitization of the guinea-pig ileum to the action of bradykinin by trypsin hydrolysate of ox and rabbit plasma, Brit. J. Pharmacol. Chemother. *32*, 453.

Aarsen, P.N. and M. van Caspel-de Bruyn, 1970, Effect of changes in ionic environment on the action of bradykinin on the guinea-pig taenia coli, European J. Pharmacol. *12*, 348.

Aarsen, P.N. and A. Kemp, 1962, Influence of metals on the activity of a bradykinin-destroying enzyme kininase, Brit. J. Pharmacol. Chemother. *19*, 442.

Abe, K., N. Watanabe, N. Kumagai, T. Mouri, T. Seki and Y. Yoshinaga, 1967, Circulating plasma kinin in patients with bronchial asthma, Experientia (Basel) *23*, 626.

Alabaster, V.A. and Y.S. Bakhle, 1972, The inactivation of bradykinin in the pulmonary circulation of isolated lungs, Brit. J. Pharmacol. Chemother. *45*, 299.

Allwood, M.J. and G.P. Lewis, 1964, Bradykinin and forearm blood flow, J. Physiol. (London) *170*, 571.

Ambache, N. and M. Rocha e Silva, 1951, Analysis of certain interactions of nicotine with bradykinin and histamine. Brit. J. Pharmacol. Chemother. *6*, 68.

Antonio, A., 1968, The relaxing effect of bradykinin on intestinal smooth muscle, Brit. J. Pharmacol. Chemother. *32*, 78.

Antonio, A. and M. Rocha e Silva, 1962, Increase of coronary flow produced by bradykinin on *in vitro* mammalian hearts, The Pharmacologist *3*, 72.

Armstrong, D., J.B. Jepson, C.A. Keele and J.W. Stewart, 1957, Pain producing substances in human inflammatory exudate and plasma, J. Physiol. (London) *135*, 350.

Auerswald, W. and W. Doleschel, 1967, On the potentiation of kinins by sulphydryl compounds, Arch. Intern. Pharmacodyn. Ther. *168*, 188.

Bauer, G., E. Zieggler and H. Konzett, 1966, Zur Hemmwirkung von Kininen an isolierten Darmpräparaten, Arch. exp. Path. u. Pharmakol. *254*, 235.

Becker, E.L. and L. Kagen, 1964, The permeability globulins of human serum and the biochemical mechanism of hereditory angioneurotic oedema, Ann. N.Y. Acad. Sci. *116*, 866.

Beloff, A. and R.A. Peters, 1945, Observations upon thermal burns: The influence of moderate temperature burns upon a proteinase of skin, J. Physiol. (London). *103*, 461.

Berry, P.A., 1966, Slow-reacting substances in anaphylaxis (SRS-A), its release, actions and antagonism. Ph.D. Thesis, C.N.A.A., London.

Berry, P.A. and H.O.J. Collier, 1964, Bronchoconstrictor action and antagonism of a slow reacting substance from anaphylaxis of guinea-pig lung, Brit. J. Pharmacol. Chemother. *23*, 201.

Bertelli, A., A. Cerrati, A.G. Perazzoli and M.A. Rossano, 1966, Attività antiflogistica del 3',5'-AMP ciclico, Attr. del Accad. med. Lombarda *21*, 601.

Bhalla, T.N., J.N. Sinha, K.K. Tangri and K.P. Bhargava, 1970, Role of catecholamines in inflammation, European J. Pharmacol. *13*, 90.

Biggs, R., R.G. Macfarlane and J. Pilling, 1947, Observations on fibrinolysis; Experimental activity produced by exercise or adrenaline, Lancet *1*, 402.

Boissonnas, R.A., S. Guttmann and P.A. Jacquenoud, 1960, Synthèse de la L-arginyl-L-prolyl-L-prolyl-glycyl-phénylalanyl-L-seryl-L-phénylalanyl-L-arginine. Distinctions entre cet octapeptide et la bradykinine, Helv. Chim. Acta *43*, 1481.

Bonta, I.L. and C.J. deVos, 1966, In: International Symposium on Vasoactive Polypeptides: Bradykinin and related kinins, ed. M. Rocha e Silva and H.A. Rothschild, p. 205. Sao Paulo: EDART.

Bonta, I.L. and C.J. deVos, 1967, Significance of the kinin system in the rat paw oedemas and drug effects on it, European J. Pharmacol. *1*, 222.

Brown, J.H., H.K. Mackey, D.A. Riggilo and N.L. Schwartz, 1968, Studies on the acute inflammatory response. II. Influence of antihistaminics and catecholamines on the formaldehyde-induced oedema, J. Pharmacol. exp. Therap. *160*, 243.

Brown, R.A. and G.B. West, 1965, Sympathomimetic amines and vascular permeability, J. Pharm. Pharmacol. *17*, 119.

Camargo, A. and S.H. Ferreira, 1971, Action of bradykinin potentiating factor (BPF) and dimercaprol (BAL) on the responses to bradykinin of isolated preparations of rat intestines, Brit. J. Pharmacol. Chemother. *42*, 305.

Chou, C.C., E.D. Frohlich and E.C. Texter, Jr., 1965, A comparative study of the effects of bradykinin, kallidin II and eledoisin on segmental superior mesenteric resistance, J. Physiol. (London) *176*, 1.

Cirstea, M., 1965, Potentiation of some bradykinin effects by thiol compounds, Brit. J. Pharmacol. Chemother. *25*, 405.

Collier, H.O.J., 1961, La bradykinine et ses antagonistes, Actualités Pharmacol. *14*, 53.

Collier, H.O.J., 1971, Prostaglandins and aspirin, Nature (London) *232*, 17.

Collier, H.O.J. and P.G. Shortley, 1960, Analgesic antipyretic drugs as antagonists of bradykinin, Brit. J. Pharmacol. Chemother. *15*, 601.

Collier, H.O.J. and P.G. Shortley, 1963, Antagonism by mefenamic acid and flufenamic acids of the bronchoconstrictor action of kinins in the guinea-pig, Brit. J. Pharmacol. Chemother. *20*, 345.

Collier, H.O.J. and W.J.F. Sweatman, 1968, Antagonism by fenamates of prostaglandin $F_{2\alpha}$ and of slow contracting substance on human bronchial muscle, Nature (London) *219*, 864.

Cotran, R.S., 1965, Delayed and prolonged vascular leakage in inflammation. II. Electron microscopic study of the vascular response after thermal injury, Am. J. Path. *46*, 589.

Cotran, R.S. and G. Majno, 1964, The delayed and prolonged vascular leakage in inflammation. I. Topography of the leaking vessels after thermal injury, Am. J. Path. *45*, 261.

Crunkhorn, P. and A.L. Willis, 1971, Cutaneous reactions to intradermal prostaglandins, Brit. J. Pharmacol. Chemother. *41*, 49.

Day, M. and J.R. Vane, 1963, An analysis of the direct and indirect actions of drugs on the isolated guinea-pig ileum, Brit. J. Pharmacol. Chemother. *20*, 150.

DePasquale, N.P. and G.E. Burch, 1968, Influence of bradykinin on isolated canine venous strips, Amer. Heart J. *75*, 630.

Di Mattei, P., 1962, Occurrence of kinins in epinephrine-induced pulmonary edema in rabbits, Arch. Intern. Pharmacodyn. Ther. *140*, 368.

Di Rosa, M. and L. Sorrentino, 1968, The mechanism of the inflammatory effect of carrageenin, European J. Pharmacol. *4*, 340.

Doleschel, W. and W. Auerswald, 1966, On the mechanism of potentiation of kinins by inhibitors of the fibrinolytic system, Experientia (Basel) *22*, 540.

Donaldson, V.H., 1968, Mechanisms of activation of C' esterase in hereditory angioneurotic edema plasma *in vitro*: Role of Hageman factor, clot promoting agent, J. Exp. Med. *127*, 411.

Edery, H., 1964, Potentiation of the action of bradykinin on smooth muscle by chymotrypsin and trypsin, Brit. J. Pharmacol. Chemother. *22*, 371.

Edery, H., 1965, Further studies on the sensitization of smooth muscle to the action of plasma kinins by proteolytic enzymes, Brit. J. Pharmacol. Chemother. *24*, 485.

Edery, H. and G.P. Lewis, 1962, Inhibition of plasma kininase activity at slightly acid pH, Brit. J. Pharmacol. Chemother. *19*, 299.

Edery, H. and G.P. Lewis, 1963, Kinin-forming activity and histamine in lymph after tissue injury, J. Physiol. (London) *169*, 568.

Elliot, D.F., E.W. Horton and G.P. Lewis, 1960a, Actions of pure bradykinin, J. Physiol. (London) *153*, 473.

Elliot, D.F., G.P. Lewis and E.W. Horton, 1960b, The structure of bradykinin, Biochem. J. *87*, 21P.

Elliot, D.F., G.P. Lewis and E.W. Horton, 1960c, The structure of bradykinin- a plasma kinin from ox blood, Biochem. Biophys. Res. Commun. *3*, 87.

Erdös, E.G., 1966, Hypotensive peptides: Bradykinin, kallidin and eledoisin, Advances in Pharmacol. *4*, 1.

Erdös, E.G., A.G. Renfrew, E.M. Sloane and J.R. Wohler, 1963, Enzymatic studies on bradykinin and similar peptides, Ann. N.Y. Acad. Sci. *104*, 222.

Erdös, E.G. and E.M. Sloane, 1962, An enzyme in human blood plasma that inactivates bradykinin and kallidins, Biochem. Pharmacol. *11*, 585.

Erdös, E.G. and J.R. Wohler, 1963a, Inhibitors of the *in vivo* enzymatic inactivation of bradykinin and kallidin, Life Sci. *2*, 270.

Erdös, E.G. and J.R. Wohler, 1963b, Inhibition *in vivo* of the enzymatic inactivation of bradykinin and kallidin, Biochem. Pharmacol. *12*, 1193.

Feldberg, W. and G.P. Lewis, 1964, The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin, J. Physiol. (London) *171*, 98.

Ferreira, S.H., 1965, A bradykinin-potentiating factor (BPF) present in the venom of *Bothrops jararaca*, Brit. J. Pharmacol. Chemother. *24*, 163.

Ferreira, S.H. and M. Rocha e Silva, 1962, Potentiation of bradykinin by dimercaptopropanol (BAL) and other inhibitors of its destroying enzyme in plasma, Biochem. Pharmacol. *11*, 1123.

Ferreira, S.H. and M. Rocha e Silva, 1969, Liberation of a bradykinin-like substance in the circulating blood of dogs by trypsin, chymotrypsin and nagarse, Brit. J. Pharmacol. Chemother. *36*, 611.

Ferreira, S.H. and J.R. Vane, 1967, The disappearance of bradykinin and eledoisin in the circulation and vascular beds of the cat, Brit. J. Pharmacol. Chemother. *30*, 417.

Ferreira, S.H., S. Moncada and J.R. Vane, 1971, Indomethacin and aspirin abolish prostaglandin release from the spleen, Nature New Biology *231*, 237.

Fishlock, D.J., 1966, Effect of bradykinin on the human isolated small and large intestine, Nature (London) *212*, 1533.

Fregnan, G.B. and A.H. Glässer, 1968, Vasodilating activity of the natural polypeptide physalaemin on hind limb and on coronary vascular beds of the dog. Comparison with eledoisin and nitroglycerin, Arch. Intern. Pharmacodyn. Ther. *171*, 435.

Frey, E.K., H. Kraut and E. Werle, 1950, Kallikrein (Padutin), Stuttgart: Enke.

Frimmer, M., 1961, Beeinflussung der Durchlässigheit von Blutcapillaren der Kaninchenhaut für Makromoleküle durch einige biogene, gefässaktive Substanzen, Naunyn-Schmiedebergs Arch. exp. Path. u. Pharmakol. *242*, 390.

Garcia Leme, J. and M. Rocha e Silva, 1965, Competitive and non-competitive inhibition of bradykinin on the guinea-pig ileum, Brit. J. Pharmacol. Chemother. *25*, 50.

Garcia Leme, J., E.E.S. Schapoval and M. Rocha e Silva, 1967, Factors influencing the development of local swelling induced in the rat's paw by macromolecular compounds and heating. In: Proc. Int. Symp. on Vaso-active Polypeptides, ed. Rocha e Silva, M. and H.A. Rothschild, p. 213-21, Sao Paulo: EDART.

Garcia Leme, J., L. Hamamura and M. Rocha e Silva, 1970, Effect of proteases and hexadimethrine bromide on the release of a bradykinin-like substance during heating (46°C) of rat paws, Brit. J. Pharmacol. Chemother. *40*, 294.

Gershon, M.D., 1967, Effects of tetrodotoxin on innervated smooth muscle preparations, Brit. J. Pharmacol. Chemother. *29*, 259.

Gladner, J.A., 1966, Potentiation of the effects of bradykinin. In: Hypotensive peptides, p. 344, ed. Erdös, E.G., N. Back and F. Sicuteri. Berlin-Heidelberg-New York: Springer.

Gladner, J.A., P.A. Murtaugh, J.E. Folk and K. Laki, 1963, Nature of peptides released by thrombin, Ann. N.Y. Acad. Sci. *104*, 47.

Gözsy, B. and L. Kátó, 1966, Delayed inflammatory response due to catecholamines and serotonin, Nature (London) *212*, 1049.

Greeff, K. and E. Moog, 1964, Vergleichende Untersuchungen über die bronchoconstrictorische und gefässconstrictorische Wirkung des Bradykinins, Histamins und Serotonins an isolierten Lungenpräparaten, Arch. exp. Path. u. Pharmakol. *248*, 204.

Graham, Jr. R.C., O.D. Ratnoff and J.M. Moses, 1965, Pathogenesis of inflammation. II. *"In vivo"* observations on the inflammatory effects of activated Hageman Factor and Bradykinin, J. Exp. Med. *121*, 807.

Habermann, E., 1966, Enzymatic kinin release from kininogen and from low-molecular compounds. In: Hypotensive peptides, p. 116, ed. Erdös, E.G., N. Back and F.F. Sicuteri, Berlin-New York-Heidelberg: Springer

Habermann, E., W. Klett and G. Rosenbusch, 1963, Partielle Reinigung und einige Eigenschaften eines Kininogens aus Rinderblut, Hoppe-Seylers Z. physiol. Chem. *332*, 121.

Halpern, B.N., 1964, Inhibition of local hemorrhagic Schwartzman reaction by a polypeptide possessing potent antiprotease activity, Proc. Soc. Exper. Biol. & Med. *115*, 273.

Herzheimer, H. and F. Stresemann, 1961, Effect of bradykinin aerosol in guinea-pig and man, J. Physiol. (London) *158*, 38P.

Hilton, S.M. and G.P. Lewis, 1956, The relationship between glandular activity, bradykinin formation and functional vasodilatation in the submandibular salivary gland, J. Physiol. (London) *134*, 471.

Holdstock, D.J., A.P. Mathias ,and M. Schachter, 1957, A comparative study of kinin, kallidin and bradykinin, Brit. J. Pharmacol. Chemother. *12*, 149.

Horton, E.W., 1963, The role of bradykinin in the peripheral nervous system, Ann. N.Y. Acad. Sci. *104*, 250.

Horton, E.W., 1969, Hypotheses on physiological roles of prostaglandins, Physiol. Rev. *49*, 122.

Jacques, R., 1965, Suppression, by α-methyl-DOPA and α-methyl-p-tyrosine, of the anti-inflammatory action of amidopyrine, Helv. Physiol. Acta *23*, 311.

James, G.W.L., 1969, The use of the *in vivo* trachea preparation of the guinea-pig to assess drug action on the lung, J. Pharm. Pharmacol. *21*, 379.

Kellermeyer, R.W., 1967, Inflammatory process in acute gouty arthritis 3. Vascular permeability-enhancing activity in normal human synovial fluid: induction by Hageman factor activators: and inhibition by Hageman factor antiserum, J. Lab. & Clin. Med. *70*, 372.

Kellett, D.N., 1965, The relationship of adrenaline and carbohydrate metabolism to inflammation. In: Proceedings International Symp. on non-steriodal anti-inflammatory drugs, ed. Garattini, S. and M.N.G. Dukes, p. 203-206. Amsterdam: Excerpta-Medica Foundation.

Khairallah, P.A. and I.H. Page, 1963, Effects of bradykinin and angiotensin on smooth muscle, Ann. N.Y. Acad. Sci. *104*, 212.

Klupp, H. and H. Konzett, 1965, Der Einfluss von Bradykinin und Kallidin auf den Druck in der Arteria pulmonalis, Arch. exp. Path. u. Pharmakol. *249*, 479.

Konzett, H. and E. Stürmer, 1960, Biological activity of synthetic polypeptides with bradykinin-like properties, Brit. J. Pharmacol. Chemother. *15*, 544.

Kovalčík, V., 1962, Effect of bradykinin on isolated coronary arteries, Nature (London) *196*, 174.

Kovalčík, V., 1963, The response of the isolated ductus arteriosus to oxygen and anoxia, J. Physiol. (London) *169*, 185.

Kukovetz, W.R. and G. Pöch, 1970, Inhibition of cyclic-3', 5'-nucleotide-phospodiesterase as a possible mode of action of papaverine and similarly acting drugs, Naunyn-Schmiedebergs Arch. Pharmakol. *267*, 189.

Lecompte, J.J., J. Troquet and A. Dresse, 1961, Stimulation médullo-surrénaliene par la bradykinine. Arch. Intern. Physiol. Biochem. *69*, 89.

Lewis, G.P., 1962, Bradykinin-biochemistry, pharmacology and its physiological role in controlling local blood flow. Sci. Basis Med. *14*, 242.

Lewis, G.P., 1970, Kinins in inflammation and tissue injury. In: Handbook of Experimental Pharmacology, *25*, 516-530. Berlin-Heidelberg-New York: Springer.

Lewis, G.P. and E. Reit, 1965, The action of angiotensin and bradykinin on the superior cervical ganglion of the cat, J. Physiol. (London) *179*, 538.

Lewis, G.P. and E. Reit, 1966, Further studies on the actions of peptides on the superior cervical ganglion and suprarenal medulla, Brit. J. Pharmacol. Chemother. *26*, 444.

Lloyd, S., 1962, Vascular responses of the rat to bradykinin, Brit. J. Pharmacol. Chemother. *19*, 503.

Macfarlane, R.G. and R. Biggs, 1948, Fibrinolysis. Its mechanism and significance, Blood *3*, 1167.

Mackay, M.E., A.A. Miles, M. Schachter and D.L. Wilhelm, 1953, Susceptibility of guinea-pig to pharmacological factors from its own serum, Nature (London) *172*, 714.

Margolis, J., 1959, Hageman factor and capillary permeability, Australian J. exper. Biol. & Med. Sci. *37*, 239.

Mashford, M.L. and R. Zacest, 1967, Effect of adrenaline on blood kinin levels and blood kininase activity in man, Circ. Res. *21*, (Suppl. 3), 183.

Mason, D.T. and K.L. Melmon, 1965, Effects of bradykinin on forearm venous and vascular resistance in man, Circ. Res. *17*, 106.

Mason, B and A.A. Miles, 1962, Globulin permeability factors without kininogenase activity, Nature (London) *180*, 587.

McKinney, G.R. and P.M. Lish, 1966, Interaction of beta-adrenergic blockade and certain vasodilators in dextran-induced rat paw oedema, Proc. Soc. Exp. Biol. Med. *121*, 494.

Melmon, K.L., M.J. Cline, T. Hughes and A.S. Nies, 1968, Kinins: possible mediators of neonatal circulatory changes in man, J. Clin. Investigation *47*, 1295.

Melmon, K.L., M.E. Webster, S.E. Goldfinger and J.E. Seegmiller, 1967, Presence of kinin in inflammatory synovial effusion from arthritides of varying etiologies, Arthritis & Rheumatism *10*, 13.

Miele, E. and G. De Natale, 1966, Modification by guanethidine and nethalide of the pressor effects of bradykinin in the rat, Arch. Intern. Pharmacodyn. Ther. *164*, 56.

Miles, A.A., 1964, Large molecular substances as mediators of the inflammatory reaction, Ann. N.Y. Acad. Sci. *116*, 855.

Möller, H., 1962, The catecholamines of skin in chloroform inflammation, Acta Dermato-Venereologica *42*, 458.

Mongar, J.L. and H.O. Schild, 1957, Effect of temperature on the anaphylactic reaction, J. Physiol. (London) *135*, 320.

Montgomery, E.H., 1968, The response of the rat duodenum to bradykinin, Proc. West. Pharmacol. Soc. *11*, 51.

Nakano, J., 1965, Effects of synthetic bradykinin on the cardiovascular system, Arch. Intern. Pharmacodyn. Ther. *157*, 1.

Nies, A.S., R.P. Forsyth, H.E. Williams and K.L. Melmon, 1968, Contribution of kinins to endotoxin shock in unanaesthetized Rhesus monkeys, Circ. Res. *22*, 155.

Northover, B.J., 1963, The permeability to plasma proteins of the peritoneal blood vessels of the mouse, and the effect of substances that alter permeability, J. Path. Bact. *85*, 361.

Northover, B.J. and G. Subramanian, 1962, A study of possible mediators of inflammatory reactions in the mouse foot, Brit. J. Pharmacol. Chemother. *18*, 346.

Ostfeld, A.M., I.F. Chapman, H. Goodell and H.G. Wolff, 1957, Studies in headache: summary of evidence concerning noxious agents active locally during migraine headache, Psychosom. Med. *19*, 199.

Picarelli, Z.P., O.B. Henriques and M.C.F. Oliveira, 1962, Potentiation of bradykinin action on smooth muscle by cysteine, Experientia (Basel) *18*, 77.

Pierce, J.V. and M. Webster, 1961, Human plasma kallidins: isolation and chemical studies, Biochem. Biophys. Res. Commun. *5*, 353.

Piper, P.J. and J.R. Vane, 1969, Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs, Nature (London) *223*, 29.

Potter, D.E. and E.J. Walaszek, 1972, Potentiation of the bradykinin response by cysteine: Mechanism of action, Arch. Intern. Pharmacodyn. Ther. *197*, 338.

Raekalli, J., 1963, Histochemical demonstration of monoamine oxidase in the earliest phase of wound healing, Nature (London) *199*, 496.

Ratnoff, O.D., 1966, Biology and pathology of initial stages of blood coagulation, Progr. in Hemat. *5*, 204.

Regoli, D. and J.R. Vane, 1964, A sensitive method for the assay of angiotensin, Brit. J. Pharmacol. Chemother. *23*, 351.

Riezen, H. van, 1966, Methixene: a non-competitive antagonist of bradykinin, J. Pharm. Pharmacol. *18*, 688.

Robison, G.A., R.W. Butcher and E.W. Sutherland, 1968, Cyclic AMP, Ann. Rev. Biochem. *37*, 149.

Rocha e Silva, M., 1960, Biochemistry and Pharmacology of bradykinin. In: Polypeptides which affect smooth muscles and blood vessels, p. 210 ed. M. Schachter, Oxford: Pergammon Press.

Rocha e Silva, M., 1964, Chemical mediators of the acute inflammatory reaction, Ann. N.Y. Acad. Sci. *116*, 899.

Rocha e Silva, M., 1970, Kinin Hormones, Springfield, Ill: C.C. Thomas.

Rocha e Silva, M. and A. Antonio, 1960, Release of bradykinin and the mechanism of production of a "thermic oedema (45°C)" in the rat's paw, Med. Exp. (Basel) *3*, 371.

Rocha e Silva, M., W. Beraldo, G. Rosenfeld, 1949, Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and by trypsin, Amer. J. Physiol. *156*, 261.

Rocha e Silva, M., A.P. Corrado and A.O. Ramos, 1960, Potentiation of the duration of the vasodilator effects of bradykinin by sympatholytic drugs and by reserpine, J. Pharmacol. exp. Therap. *128*, 217.

Rocha e Silva, M. and J. Garcia Leme, 1963, Antagonists of bradykinin, Med. Exp. (Basel) *8*, 287.

Rocha e Silva, M. and J. Garcia Leme, 1964, On some antagonists of bradykinin, Arch. exp. Path. u. Pharmakol. *250*, 167.

Rocha e Silva, M. and J. Garcia Leme, 1965, Studies on the antagonists of bradykinin. In: Non-steroidal anti-inflammatory drugs, p. 120, ed. Garattini, S. and M.N.G. Dukes, Amsterdam: Excerpta Medica Foundation.

Rocha e Silva, M. and S.M. Rosenthal, 1961, Release of pharmacologically active substances from the rat skin *in vivo* following thermal injury, J. Pharmacol. exp. Therap. *132*, 110.

Rosas, R., D. Montague, M. Gross and D.F. Bohr, 1965, Cardiac action of vaso-active polypeptides in the rat. I. Bradykinin, Circ. Res. *16*, 150.

Schachter, M., 1968, Physiology Society Symposium: Vasoactive peptides, Fed. Proc. *27*, 49.

Sherman, W.T. and R.F. Gautieri, 1969, Cardiovascular and gastrointestinal effects of bradykinin and its potentiation by thiols in rats, J. Pharm. Sci. *58*, 971.

Skinner, N.S. Jr. and W.J. Powell Jr., 1967, Action of oxygen and potassium on vascular resistance of dog skeletal muscle, Amer. J. Physiol.*212*, 533.

Skinner, N.S. Jr. and M.E. Webster, 1968, Kinins, beta-adrenergic receptors and functional vasodilatation in the submaxillary gland of the cat, J. Physiol. (London) *195*, 505.

Smith, J.B. and A.L. Willis, 1971, Aspirin selectively inhibits prostaglandin production in human platelets, Nature New Biology *231*, 235.

Somlyo, A.P. and A.V. Somlyo, 1970, Vascular smooth muscle II. Pharmacology of normal and hypertensive vessels, Pharmacol. Rev. *22*, 249.

Spector, W.G., 1964, Endogenous inflammatory mechanism in the rat. In: Intern. Symp. on Injury, Inflammation and Immunity, ed. Thomas, L., J.W. Uhr and L. Grant, p. 178. Baltimore: Williams and Wilkins.

Spector, W.G. and D.A. Willoughby, 1960, Suppresion of increased capillary permeability in injury by monoamine oxidase inhibitors, Nature (London) *186*, 162.

Spector, W.G. and D.A. Willoughby, 1964, Effect of vascular permeability factors on emigration of leukocytes, J. Path. Bact. *87*, 341.

Spector, W.G., M. N-I. Walters and D.A. Willoughby, 1965, Venular and capillary permeability in thermal injury, J. Path. Bact. *90*, 635.

Starr, M.S. and G.B. West, 1967, Bradykinin and oedema formation in heated paws of rats, Brit. J. Pharmacol. Chemother. *31*, 178.

Stürmer, E. and A. Cerletti, 1961, Bradykinin, Amer. Heart J. *62*, 149.

Sutter, M.C., 1965, The pharmacology of isolated veins, Brit. J. Pharmacol. Chemother. *24*, 742.

Tewksbury, D.A., 1968, Studies on the mechanism of bradykinin potentiation, Arch. Intern. Pharmacodyn. Ther. *173*, 426.

Trautschold, I., 1968, In: Das Kallikrein-Kinin System und seine Inhibitoren, ed. Frey, E.K., H. Kraut, E. Werle, R. Vogel, G. Zickgraf-Rüdel and I. Trautschold, p. 223, Stuttgart: Enke.

Trautschold, I., 1970, Assay methods in the kinin system. In: Handbook of Experimental Pharmacology, *25*, 52-81, Berlin-Heidelberg-New York: Springer.

Trendelenburg, U., 1966, Observations on the ganglion-stimulating action of angiotensin and bradykinin, J. Pharmacol. exp. Therap. *154*, 418.

Triner, L., Y. Vulliemoz, I. Schwartz and G.G. Nahas, 1970, Cyclic phosphodiesterase activity and the action of papavarine, Biochem. Biophys. Res. Commun. *40*, 64.

Türker, K.R. and P.A. Khairallah, 1966, Action of eledoisin on isolated vascular strips, Experientia (Basel) *22*, 696.

Türker, K.R., B.K. Kiran and S. Kaymakçalan, 1964, The effects of synthetic bradykinin on intestinal motility in different laboratory animals and its relation to catecholamines, Arch. Intern. Pharmacodyn. Ther. *151*, 260.

Ungar, G., 1947, Release of proteolytic enzymes in anaphylactic and peptone shock *in vitro*, Lancet *I*, 708.

Vane, J.R., 1971, Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs, Nature New Biology *231*, 232.

Vargaftig, B.B. and N. Dao, 1971, Release of vasoactive substances from guinea-pig lungs by slow-reacting substance C and arachidonic acid, Pharmacology *6*, 99.

Vargaftig, B.B., E.P. de Miranda and B. Lacoume, 1969, Inhibition by non-steroidal anti-inflammatory agents of *in vivo* effects of "slow reacting substance C", Nature (London) *222*, 883.

Vogt, W., 1966, Demonstration of the presence of two separate kinin-forming systems in human and other plasma. In: Hypotensive peptides, p. 185, ed. by E.G. Erdös, N. Back and F. Sicuteri, Berlin-Heidelberg-New York: Springer.

Vogt, W., G. Garbe and G. Schmidt, 1967, Untersuchungen zur Existanz zweir verschiedene kininbildener Systems in menschlichem plasma Naunyn-Schmiederbergs Arch. exp. Path. Pharmak. *256*, 127.

Walaszek, E.J., C.G. Huggins and C.M. Smith, 1963, Drugs that modify actions of pharmacologically active polypeptides, Ann. N.Y. Acad. Sci. *104*, 281.

Warren, K.S. and R.W. Kellermeyer, 1968, Foreign body granuloma as a response to chemical mediators of inflammation, J. Clin Investigation *47*, 99.

Wells, F.R. and A.A. Miles, 1963, Site of the response of thermal injury, Nature (London) *200*, 1015.

Werle, E., 1934, Über die Inaktivierung des Kallikreins. II. Mitt. Biochem. Z. *273*, 291.

Werle, E., 1937, Über die Wirkung des Kallikreins auf den isolierten Darm und über eine neue darmkontrahierende Substanz, Biochem. Z. *289*, 217.

Werle, E., 1955, The chemistry and pharmacology of kallikreins and kallidin. In: Polypeptides which stimulate plain muscle, ed. Gaddum, J.H., Edingburgh: J.H. Livingstone.

Werle, E., 1963, Plasma kinins, Munich med. Wsch. *50*, 2846.

Werle, E., I. Trautschold and G. Leysath, 1961, Isolierung und Struktur des Kallidins, Z. physiol. Chem. *326*, 174.

Wiegershausen, B., G. Stopp and M. Eichstädt, 1964, Zum Mechanismus der Bradykininwirkung am Meerschweinschendarm, Acta Biol. Med. German. *12*, 443.

Wiegershausen, B., G. Rostock and I. Paegelow, 1973, Der Einfluss einiger Lokalanaesthetika auf die erregende Wirkung von Bradykinin am glatten Muskel. III. Mitt., Arch. Intern. Pharmacodyn. Ther. *201*, 106.

Wilhelm, D.L., 1962, The mediation of increased vascular permeability in inflammation, Pharmacol. Rev. *14*, 251.

Wilhelm, D.L. and B. Mason, 1960, Vascular permeability changes in inflammation: role of endogenous permeability factors in mild thermal injury, Brit. J. Exp. Path. *41*, 487.

Willis, A.L., 1969, In: Prostaglandins, peptides and amines, ed. Mantegazza, P. and E.W. Horton, London: Academic Press.

Willoughby, D.A. and W.G. Spector, 1964, Adrenaline precursors in the inflammatory reaction, J. Path. Bact. *88*, 159.

Zacariae, H., J. Malmquist, J.A. Oates and W. Pettinger, 1967, Studies on the mechanism of kinin formation in inflammation, J. Physiol. (London) *190*, 81.

Zweifach, B.W., 1966, Microcirculatory effects of polypeptides. In: Hypotensive peptides, ed. Erdös, E.G., N. Back and F. Sicuteri, p. 451, Berlin-Heidelberg-New York: Springer.

Chapter 2

THE BIPHASIC RESPONSE OF THE ISOLATED GUINEA-PIG ILEUM BY BRADYKININ

SUMMARY

The isolated guinea-pig ileum, challenged by spasmogens, was used to study the effect of bradykinin. In the presence of acetylcholine producing approximately 60 % of maximum contraction, bradykinin caused relaxation followed by contraction. The biphasic response to bradykinin was also found in the presence of histamine, eledoisin, angiotensin, prostaglandin $F_{2\alpha}$ and transmural electrical stimulation. The conditions for bradykinin-induced relaxation were not found after contraction by bradykinin, and potassium or barium chloride. Under conditions where bradykinin produced a biphasic response, acetylcholine, histamine, eledoisin, angiotensin, prostaglandin $F_{2\alpha}$ and lysine vasopressin only contracted the ileum, while adrenaline, noradrenaline, oxytocin, calcium and magnesium chloride only relaxed. On increasing the percentage of maximum contraction of the ileum with acetylcholine, a linear inverse relationship with relaxation by bradykinin was found. Tachyphylaxis was not present with the bradykinin-induced relaxation. The relaxing effect of bradykinin is more likely to be due to a direct action on the muscle cell membrane than to a release of a mediator or to blockade of a receptor mediating contraction.

INTRODUCTION

Bradykinin has been shown to produce a slow contraction of many intestinal smooth muscle preparations, including the rat fundus strip, rabbit duodenum and large intestine, and the guinea-pig ileum (Rocha e Silva, 1970; Trautschold, 1970). The mechanism for the guinea-pig ileum has been thought to represent a direct action of the kinin on the smooth muscle (Day and Vane, 1963; Khairallah and Page, 1963; Wiegershausen et al., 1964; Gershon, 1967) and also indirectly via the release of acetylcholine (Wiegershausen et al., 1964). The former

mechanism was proposed since no other indirect mechanism could be demonstrated and further that tetrodotoxin, eserine and hyoscine did not affect the maximal response with bradykinin. The latter indirect mechanism was the result of the finding that contraction of the isolated guinea-pig ileum could be inhibited by atropine and morphine, and potentiated by eserine. The inhibition was manifested by a reduction and the potentiation by an increase in the contraction speed of the organ.

Apart from the contractile effects of bradykinin on intestinal smooth muscle, it is also known that the rat duodenum is relaxed by bradykinin (Elliot et al., 1960; Antonio, 1968). The effect on this organ is thought to be one of the characteristic features of the pharmacology of bradykinin (Rocha e Silva, 1970), however indications have been found in the literature which suggest that bradykinin can also relax the rabbit duodenum and rat colon (Elliot et al., 1960), the human small and large intestine (Fishlock, 1966), the guinea-pig taenia coli (Regoli and Vane, 1964), the hen rectal caecum (Erdös, 1966) and the rat ileum (Sherman and Gautieri, 1969). These results stress the importance of not only considering the contractile effects of bradykinin on smooth muscle, but also a possible ubiquitous relaxation component before the contraction by bradykinin on smooth muscle.

To our knowledge relaxation of the guinea-pig ileum by bradykinin has never been previously demonstrated. We observed this response first during experiments designed to establish a relationship between cyclic-3', 5'-AMP and the bradykinin contraction. In an attempt to stimulate the action of phosphodiesterase the ileum was preincubated with imidazole (Robison et al., 1968), which caused a sustained contraction of the ileum. Addition of bradykinin resulted in a biphasic response, first a relaxation, then a contraction. The relaxation provoked considerable interest and was accordingly quantified, but using acetylcholine in place of imidazole to contract the ileum. In this chapter are described some conditions under which the guinea-pig ileum can be relaxed by bradykinin, and also some of the properties of this relaxation.

MATERIALS AND METHODS

Female guinea-pigs weighing between 200-250 g were sacrificed by decapitation and bleeding. The abdominal cavity was exposed and the entire small intestine removed. The terminal ileum, taken to be the 10 cm immediately before the caecum, was discarded and a piece of ileum approximately 2 cm long adjacent to the terminal section was washed in warm Tyrode solution. Taenia coli strips of 2 cm length were taken from the large intestine. The organs were suspended in a 5-ml organ bath at 37°C, and gassed with air. Changes in the length of the preparations were detected by a Harvard Heart/Smooth Muscle Transducer (Model 356) with a total load on the organ of 0.5 g and recorded by a Harvard Electronic Recording Module (Model 350). Two platinum electrodes were arranged for transmural stimulation, when required, and stimulation was given by a Grass Model S 6 stimulator, which had been converted to give a low frequency of 40mHz in place of 2Hz.

The Tyrode solution had the following composition g/l in deionized water: NaCl 8.00, KCl 0.20, $CaCl_2$ 0.20, $MgCl_2$ 0.10, $NaHCO_3$ 1.00, NaH_2PO_4 0.05, glucose 1.00. The following drugs were used: synthetic bradykinin (Sandoz, Switzerland) prepared in 10 µg freeze-dried samples (Organon, Holland) with a binding medium of the following composition: toluene-4-sulphonic acid 0.1 mg, gelatin (Hydrolysate) 1 mg, mannitol 15 mg; acetylcholine chloride (Merck, Germany); histamine dichloride, l-adrenaline bitartrate, l-noradrenaline hydrochloride (Fluka, Switzerland); angiotensin (Hypertensin^{R}, CIBA-Geigy, Switzerland), Prostaglandin $F_{2\alpha}$ was kindly donated by Upjohn U.S.A., synthetic oxytocin and lysine vasopressin by Organon, Holland and eledoisin trifluoracetate by Sandoz, Switzerland. All drugs were dissolved in Tyrode solution and doses refer to their salts. Other chemicals used were of analytical grade.

Dose-response relationships

In the experiments to be described acetylcholine was added at a concentration of between 10-20 ng/ml, depending on the sensitivity and length of the ileum to produce approximately 60 % of the maximum contraction, and 1 min thereafter was added bradykinin at a concentration ranging between 1-100 ng/ml depending on the circumstances of the experiment. Bradykinin was allowed to remain in contact with the organ for 40 sec before washing out. Further additions were made in 4-min cycles. At low concentrations of bradykinin (1-4 ng/ml) a linear relaxation dose-response curve could be constructed (figs. 1 and 2), the response being measured from the elevated base line to

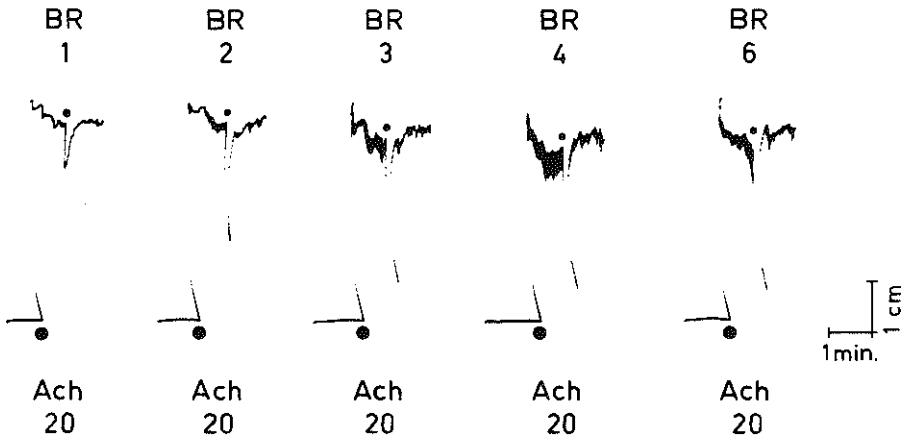

Fig. 1. Effect of increasing doses of bradykinin (BR) on the guinea-pig ileum added 1 min after doses of acetylcholine (Ach) producing about 60% maximum contraction. Bradykinin was in contact with the ileum 40 sec before washing out. Numbers relate to ng/ml.

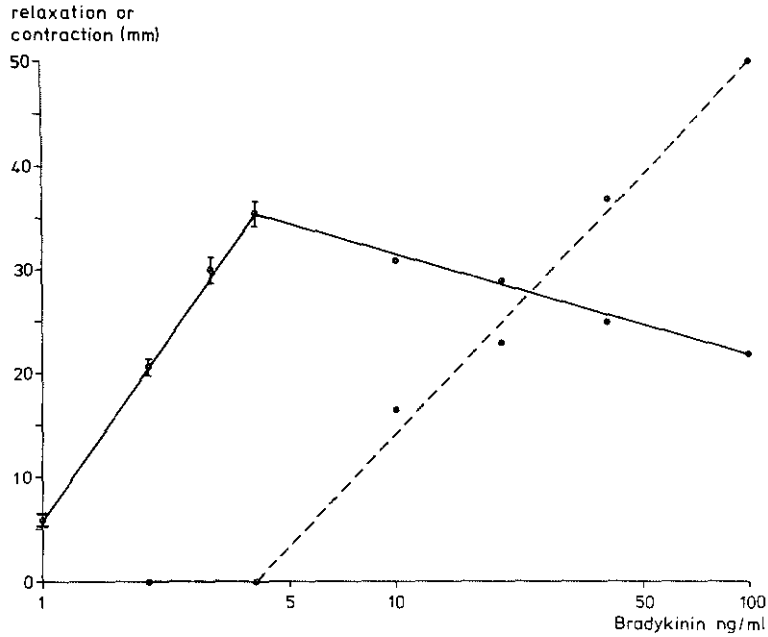

Fig. 2. Dose-response relationships for the relaxation (solid line) and contraction (broken line) of the guinea-pig ileum by bradykinin added 1 min after the addition of acetylcholine, 20 ng/ml, producing about 60% maximum contraction. Points with a vertical line (S.E.M.) represent 8 experiments, while other points are the mean of two experiments. Relaxation and contraction responses were measured at the same time on the same ileum after acetylcholine.

the deepest point of the relaxation. At concentrations of bradykinin above 4 ng/ml the relaxation dose-response curve became non-linear, and as can be seen from fig. 2 contractions were produced at concentrations greater than 4 ng/ml.

Specificity of the bradykinin relaxation

It was considered that the relaxation of the acetylcholine contracted guinea-pig ileum may not be a specific property of bradykinin. To this end some biologically active substances were examined under the same conditions. These results are shown in fig. 3. Both noradre-

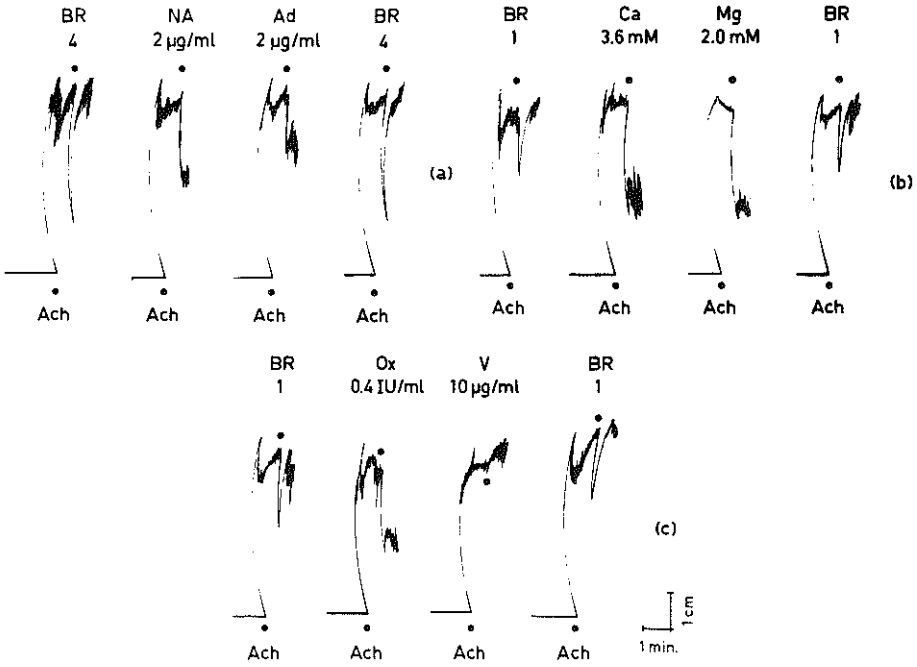

Fig. 3. Effect of bradykinin (BR), noradrenaline (NA), adrenaline (Ad), calcium chloride (Ca), magnesium chloride (Mg), oxytocin (Ox) and lysine vasopressin (V) on the guinea-pig ileum, added 1 min after doses of acetylcholine (Ach) producing about 60% maximum contraction. Acetylcholine was in contact with the ileum 1 min 40 sec before washing out. Numbers relate to ng/ml, unless specified.

naline and adrenaline (2µg/ml) relaxed the acetylcholine contracted ileum, but differed from bradykinin in that their relaxations were maintained i.e. there was no recovery from the relaxation to the elevated base line (fig. 3a). A similar result was found with 3.6 mM Ca^{2+} and 2.0 mM Mg^{2+} (fig. 3b). Oxytocin produced only a relaxation at 400 mIU/ml, while lys-vasopressin produced a small contraction at 10 µg/ml (fig. 3c). Acetylcholine, 1-20 ng/ml, histamine, 1-20 ng/ml, eledoisin, 1-200 ng/ml, prostaglandin $F_{2\alpha}$, 200-1000 ng/ml and angiotensin, 1-200 ng/ml produced only contraction responses after the standard contraction with acetylcholine. The carrier medium for bradykinin produced no effect at 100 times the normal bath concentration. The latter figures are not included for the sake of brevity. Initial

investigation of the guinea-pig taenia coli has brought to light a different result with angiotensin (fig. 4). It can be seen on increasing the angiotensin concentration between 4-400 ng/ml the appearance of a biphasic response. It can also be observed that the taenia coli is 10 or more times less active towards relaxation by angiotensin, while 10 or more times more active to contraction by angiotensin than bradykinin.

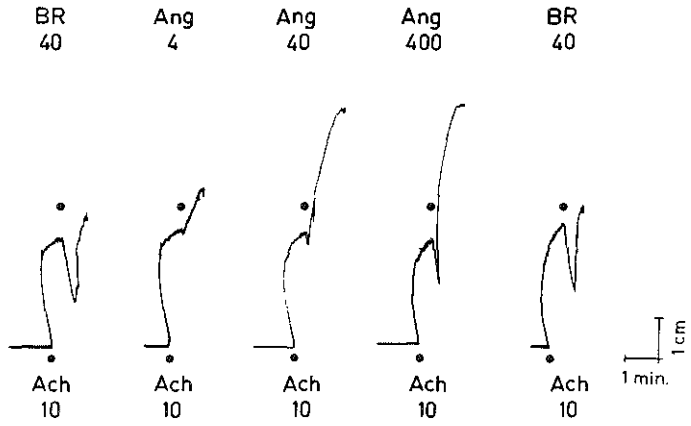

Fig. 4. Effect of bradykinin (BR) and angiotensin (Ang) added 1 min after acetylcholine (Ach), 10 ng/ml, contractions of the guinea-pig taenia coli. Acetylcholine was in contact with the taenia coli 1 min 40 sec before washing out. Numbers relate to ng/ml.

Conditions for the bradykinin relaxation

These experiments were designed to show whether the relaxation response is dependent on any one particular type of receptor. Various spasmogens of a dissimilar nature were given to the ileum in place of acetylcholine, the doses chosen such that an approximately 60 % maximum contraction of the spasmogen contraction was obtained. By this means conditions imitating acetylcholine were achieved (Fig. 5). It can be observed that histamine at doses of 40 ng/ml and angiotensin, 2 ng/ml, provided the necessary conditions for relaxation (fig. 5a). After histamine the relaxation was similar, but after angiotensin it was reduced and a contraction became evident. With eledoisin, 2 ng/ml, and prostaglandin $F_{2\alpha}$, 500 ng/ml, the conditions for the relaxation

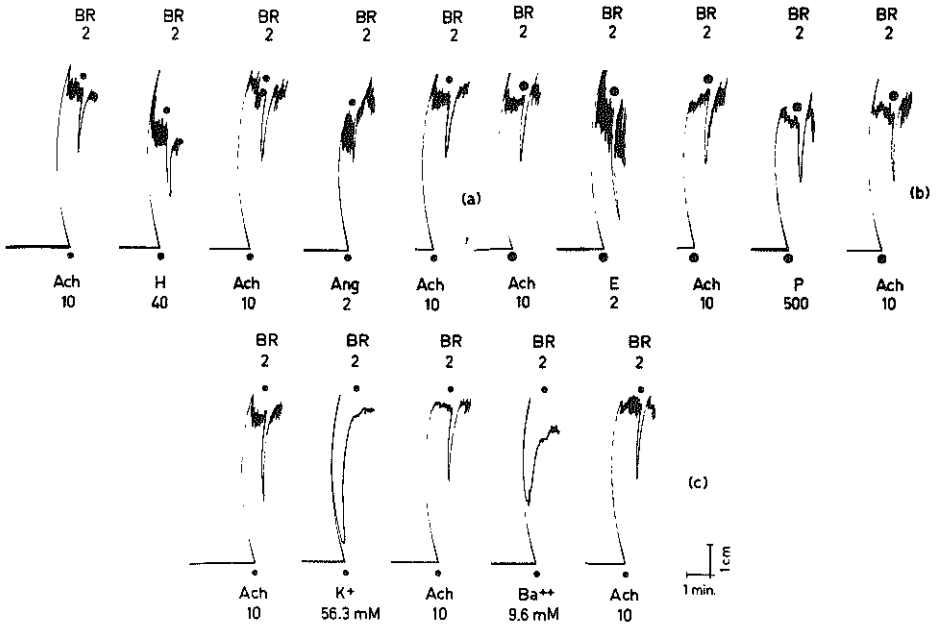

Fig. 5. Effect of a standard dose of bradykinin (BR) on the guinea-pig ileum, added 1 min after acetylcholine (Ach), histamine (H), angiotensin (Ang), eledoisin (E) and prostaglandin $F_{2\alpha}$ (P), potassium chloride (K^+) and barium chloride (Ba^{++}) producing approximately the same percentage of maximum contraction as acetylcholine. Bradykinin was in contact with the ileum 40 sec before washing out. Numbers relate to ng/ml, unless specified.

were also met (fig. 5b), however with eledoisin the bradykinin relaxation was larger than that after the standard acetylcholine contraction. This observation could be repeatedly demonstrated using different ilea. When potassium chloride, 56.3 mM, or barium chloride, 9.6 mM, was in place of acetylcholine (fig. 5c), the ileum displayed an initial spike followed by a slow maintained contraction. No relaxation was found with bradykinin after either, but in their place was a small contraction. Similar results were also found with 125.4 mM and 158.0 mM potassium chloride. In the case of the transmurally stimulated ileum (20V, 10 msec, 0.36 Hz) increasing doses of bradykinin in the range 0.5 - 3.0 ng/ml resulted in increasing relaxations (fig. 6). A concentration of 4 ng/ml did not produce a linear response, and on occasions a small contraction resulted.

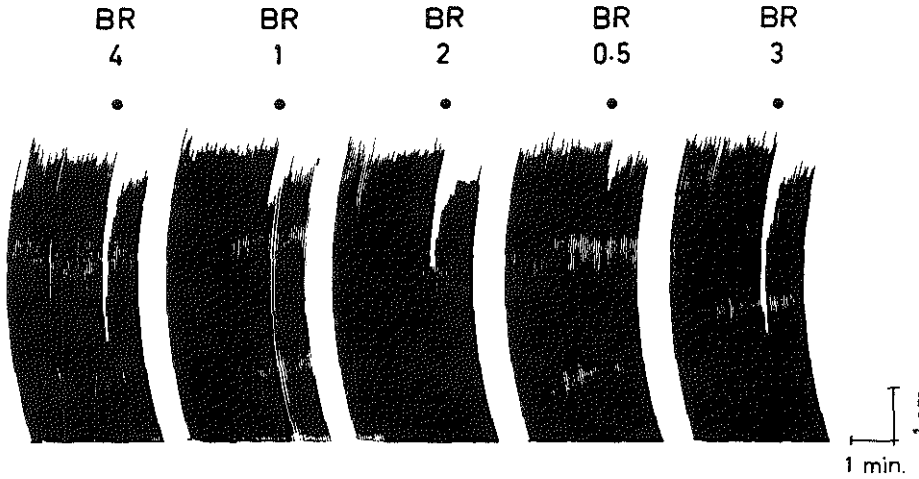

Fig. 6. Effect of various doses of bradykinin (BR) added to the transmurally stimulated guinea-pig ileum. Stimulation was at 20 V, 10 msec, 0.36 Hz. Bradykinin was in contact with the ileum 40 sec before washing out. Numbers relate to ng/ml.

Variation in acetylcholine concentration

If the concentration of challenging acetylcholine was increased then the elevated base line also increased. On raising the acetylcholine base line, which is conveniently expressed as the percentage of the maximum acetylcholine contraction, the relaxation produced by bradykinin can be seen to decrease (table 1). The equations for the variation in the percentage of maximum contraction with acetylcholine and the bradykinin relaxation are depicted in fig. 7. Calculations using these regression equations are found in subsequent chapters. The sensitivity of the recording module was constant throughout the experiments, however the base line for measurement of contraction was adjusted by vertical displacement of the transducer at each acetylcholine concentration, thus allowing the relaxations to be measured on the chart. The maximal contraction can be estimated in the region of 125 mm at this sensitivity.

Table 1

The effect of the increase in acetylcholine concentration and percentage of maximal contraction of the guinea-pig ileum on the relaxation produced by a constant dose of bradykinin.

Acetylcholine concentration ng/ml	Maximal contraction ± S.E.M. (%)	Bradykinin*relaxation ± S.E.M. (mm)
10	28 ± 1	43.0 ± 0.7 (6)**
20	63 ± 3	21.6 ± 1.2 (11)
40	85 ± 4	8.8 ± 0.6 (5)
100	100 ± 2	1.9 ± 0.2 (5)
200	100 ± 2	1.0 ± 0.0 (5)

* Bradykinin, 2 ng/ml

** Numbers of experiments in parenthesis. The correlation coefficient (r) between the $\log_{10}$ acetylcholine concentration and the bradykinin relaxation was - 0.923. The linear regression equation was $y = -0.16x_1 + 27.36$, where y = bradykinin relaxation (mm) and $x_1 = \log_{10}$ acetylcholine concentration. The correlation coefficient (r) between the % maximal contraction and the bradykinin relaxation was - 0.999. The linear regression equation was $y = -0.58x + 58.62$, where y = bradykinin relaxation (mm) and x = % maximal contraction.

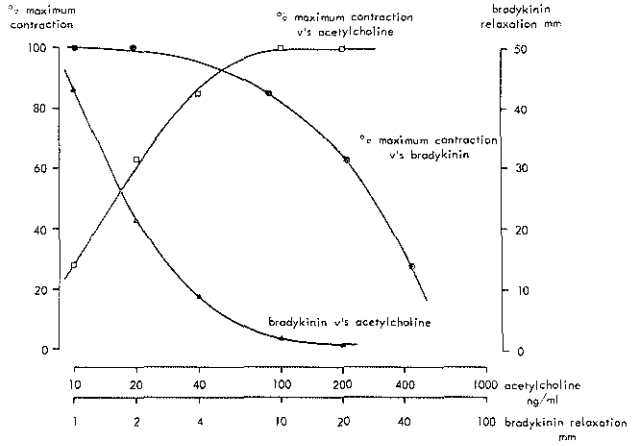

Fig. 7. Effect of varying the challenging acetylcholine concentration, and the percentage of maximum contraction using acetylcholine on the bradykinin relaxation (2 ng/ml) of the guinea-pig ileum.
Open squares: Percentage of maximum contraction plotted against acetylcholine concentration.
Solid triangles: Bradykinin relaxation plotted against acetylcholine concentration.
Solid circles: Percentage of maximum contraction plotted against bradykinin relaxation.

Table 2

The effect of bradykinin pretreatment on the bradykinin relaxation of the acetylcholine contracted guinea-pig ileum.

Bradykinin added*	Bradykinin relaxtion**	
(ng/ml)	Controls mm ± S.E.M.	Bradykinin pretreated mm ± S.E.M.
0.1	25.9 ± 1.1	19.7 ± 1.0 (5)$^{\Delta}$
0.2	25.0 ± 0.8	16.3 ± 1.2 (5)$^{\Delta}$
1.0	24.8 ± 1.1	9.6 ± 0.3 (5)$^{\Delta}$
2.0	25.4 ± 2.3	6.0 ± 0.9 (7)$^{\Delta}$

* Bradykinin was added 1 min before acetylcholine (20 ng/ml).

** Bradykinin, 2 ng/ml.

Δ Numbers of experiments in parentheses . $p \leq 0.01$ (Student's t-test). The correlation coefficient (r) between the $\log_{10}$ bradykinin concentration and the bradykinin relaxation was - 0.999.

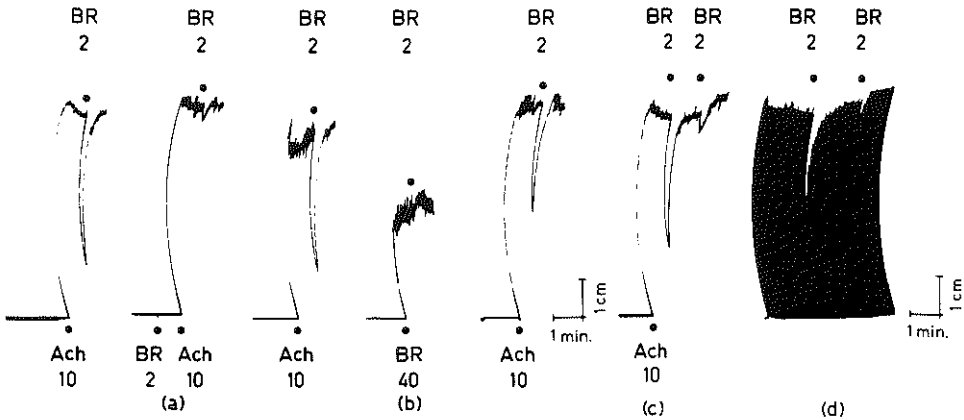

Fig. 8. Pretreatment of the guinea-pig ileum by bradykinin (BR);
a) Effect of a non-contracting dose of bradykinin added 1 min before a dose of acetylcholine (Ach), 10 ng/ml, on the relaxation produced by bradykinin 1 min after the acetylcholine.
b) Effect of a contracting dose of bradykinin, 40 ng/ml, on the relaxation produced by a further non-contracting dose of bradykinin in the absence of acetylcholine.
c) Effect of cumulative sub-maximal relaxation doses of bradykinin, added after the standard acetylcholine contraction.
d) Effect of cumulative sub-maximal relaxation doses of bradykinin added during transmural stimulation, 20 V, 10 msec, 0.36 Hz.
Numbers relate to ng/ml.

Action of bradykinin on its own response

Addition of bradykinin 1 min before the standard acetylcholine contraction resulted in a reduction of the subsequent bradykinin relaxation (fig. 8a). On varying the concentration of bradykinin in the range 0.1-2.0 ng/ml there resulted a dose dependent relationship where the correlation coefficient (r) was 0.999 (table 2). When bradykinin (40 ng/ml) was given to the ileum in place of acetylcholine there was a sustained contraction (fig. 8b). The relaxation of a further dose of bradykinin (2 ng/ml) was prevented and in its place a small contraction was observed. Further, if a dose of bradykinin giving sub-maximal relaxation responses was given 1 min after a standard acetylcholine contraction and bradykinin, 2 ng/ml (fig. 8c) or 1.5 min after a sub-maximal relaxation dose of bradykinin, 2 ng/ml, on the transmurally stimulated ileum (fig. 8d), the relaxation response of bradykinin was seen to be greatly diminished.

DISCUSSION

In the system used for this investigation it is possible to record both an initial relaxation and a further contraction. At lower doses of bradykinin 1-4 ng/ml a dose-response curve could be constructed, but at higher concentrations the curve became non-linear and even decreased. At a threshold concentration of 4 ng/ml for these preparations a contraction response became evident. From the experiments described in this chapter the sensitivity of the bradykinin relaxation was apparently of the same order as that of the contraction of the rat uterus or the relaxation of the rat duodenum (Rocha e Silva, 1970).

The relaxation found with bradykinin on the guinea-pig ileum when compared to that of adrenaline, noradrenaline, calcium, magnesium or oxytocin shows a remarkedly different form. With the latter substances the guinea-pig ileum responds only with relaxation, without the concomitant return to the original elevated base line, or with larger concentrations of bradykinin, contractions. Of the other biologically active substances examined only contraction responses were found. These results indicate that the property of relaxation, followed by contrac-

tion of the acetylcholine contracted guinea-pig ileum is probably specific to bradykinin.

Similarities in the actions of bradykinin and angiotensin have been previously reported on the superior cervical ganglion of the cat (Lewis and Reit, 1965) and the central nervous system of the cat (Čapek et al., 1969). The finding that angiotensin first relaxes then contracts the guinea-pig taenia coli at concentrations greater than 40 ng/ml suggests there may be a similarity of action of these peptides in giving relaxation of this organ. It can also be seen that for relaxation bradykinin is about 40 times more active on the ileum than on the taenia coli. These results are however only qualitative, a more detailed investigation being necessary before a full comparison of these peptides can be made.

It appears possible to use not only acetylcholine as the initial challenging spasmogen but also histamine, eledoisin, angiotensin and prostaglandin $F_{2\alpha}$. After angiotensin there results a smaller relaxation with bradykinin, than after acetylcholine. An explanation might be that if angiotensin has a slight bradykinin-like activity, but not enough to elicit a visual relaxation of the guinea-pig ileum, it may still function like bradykinin in inhibiting its own relaxation response. After eledoisin, bradykinin can be seen to produce a larger relaxation than after acetylcholine. No explanation can be given for these phenomena at the present time but these questions are further discussed in chapter 4. Transmural stimulation also provides the conditions for relaxation of the guinea-pig ileum. Evidence suggests that this stimulation contracts the organ via excitation of the postganglionic cholinergic fibres and also via a direct action on the effector cell structures (Paton, 1955). From these combined results it would appear improbable that bradykinin is having any transient blocking activity on any of the known receptors, since no common receptor is involved in contraction by any of the previous spasmogens used.

Smooth muscle depolarized by potassium rich solutions can be contracted and relaxed by drugs, the responses being uncomplicated by their effects on membrane depolarization and electrical conduction (Schild, 1964). The guinea-pig ileum when treated with the various potassium concentrations undergoes first a brief contraction followed

by relaxation, then forms a maintained elevated base line. The maintained contraction is considered to be dependent on the calcium content of the bathing medium (Durbin and Jenkinson, 1961). A fully depolarizing potassium concentration was taken to be 158 mM. This concentration is within the range used by various authors (Durbin and Jenkinson, 1961; Edman and Schild, 1963). No concentrations of potassium chloride examined gave the correct conditions for the bradykinin relaxation, even though the lower concentrations were not fully depolarizing. It can also be observed that increasing the degree of contraction of the ileum with acetylcholine gives a progressive reduction in the bradykinin relaxation and so does not provide the correct conditions. These results were found to have a correlation coefficient (r) of - 0.999, suggesting an almost perfect inverse linear relationship between the two parameters. It is possible that increasing the acetylcholine concentration to give maximal contractions produces a similar effect as depolarizing concentrations of potassium chloride. These results with increasing concentrations of acetylcholine and depolarizing potassium chloride solutions can be taken in conjunction with those of Antonio (1968) on the isolated rat duodenum. When he used 42 mM potassium chloride he found that the bradykinin relaxation was eliminated and in its place was a small contraction which was unrelated to the dose of bradykinin. In the present experiments higher concentrations were found to give a similar result. He also found a concentration of acetylcholine, 1 µg/ml, reduced the bradykinin relaxation at calcium concentrations between 0.25 - 8.0 mM. This acetylcholine concentration is 10 times that which almost completely eliminates the bradykinin relaxation on the guinea-pig ileum. Barium ions were thought to contract intestinal smooth muscle indirectly through the cholinergic nerve elements (Innes et al., 1957), but barium contractions have been recently shown to be insensitive to atropine, hemicholinium and hexamethonium (Shibata et al., 1970). It seems probable that barium induced contractions are mediated by membrane potential changes such as depolarization (Suzuki et al., 1964), and that there is a specific site of action for barium ions (Takagi and Takayanagi, 1962). In the former respect the action of barium resembles that of potassium ions in depolarizing the ileum. These results suggest that bradykinin has no influence on the processes mediating drug-induced

contraction in the presence of fully depolarizing concentrations of K^+ or Ba^{++}, but rather has a direct influence on the properties of the muscle cell membrane. Bradykinin can be thought of to act on either the inhibition of conducted impulses or spike activity or both. Recent work on the guinea-pig taenia coli (Aarsen and Van Caspel-de Bruyn, 1970) using the sucrose gap technique showed that bradykinin mainly suppressed spike activity, but also exhibited very small hyperpolarizations. They implicate Ca^{2+}, since under normal conditions these ions have been found to carry most of the current during the action potential (Bülbring and Kuriyama, 1963).

The necessary conditions for relaxation were also not provided by a contracting dose of bradykinin. Not only did this dose of bradykinin not provide the correct conditions, but also non-contracting doses given before a constant amount of acetylcholine prevented the relaxation response in a dose-dependent manner. It can also be seen that a submaximal dose of bradykinin given after acetylcholine prevented the relaxation produced by a similar dose. This was found irrespective of the fact that the ileum was challenged first by acetylcholine or stimulated transmurally. In the light of the definition for tachyphylaxis (Bowman et al., 1969), one can see that the conditions are not satisfied, since bradykinin is not washed out after each addition. Also it can be said that at no time during the course of an experiment or series of experiments on the same ileum were there diminishing bradykinin relaxation responses observed. It is thus unlikely that bradykinin is releasing a mediator from stores e.g. catecholamines. Continual release of a stored mediator by bradykinin over a period of hours should ultimately results in a diminished response, which is not found. If a receptor exists for the relaxation with bradykinin, then it appears that bradykinin has the ability to either saturate the receptor at very low concentrations or to alter the confirmation of the membrane as to preclude further binding of the bradykinin to the existing free receptors. The latter hypothesis seems more likely, since even sub-maximal doses can be shown to antagonize each other, where there is unlikely to be full receptor saturation.

REFERENCES

Aarsen, P.N. and M. van Caspel- de Bruyn, 1970, Effect of changes in ionic environment on the action of bradykinin on the guinea-pig taenia coli, European J. Pharmacol. *12*, 348.

Antonio, A., 1968, The relaxing effect of bradykinin on intestinal smooth muscle, Brit. J. Pharmacol. Chemother. *32*, 78.

Bowman, W.C., M.J. Rand and G.B. West, 1969, In: Textbook of Pharmacology, p. 478, Oxford: Blackwell Scientific Publications.

Bülbring, E. and H. Kuriyama, 1963, Effect of changes in the external sodium and calcium concentrations on spontaneous electrical activity in smooth muscle of guinea-pig taenia coli, J. Physiol.(London) *166*, 29.

Čapek, R., K. Mašek, M. Šramka, M. Kršiak and P. Švec, 1969, The similarities of the angiotensin and bradykinin action on the central nervous system, Pharmacology *2*, 161.

Day, M. and J.R. Vane, 1963, An analysis of the direct and indirect actions of drugs on the isolated guinea-pig ileum, Brit. J. Pharmacol. Chemother. *20*, 150.

Durbin, R.P. and D.H. Jenkinson, 1961, The effect of carbachol on the permeability of depolarized smooth muscle to inorganic ions, J. Physiol. (London), *157*, 74.

Edman, K.A.P. and H.O. Schild, 1963, Calcium and the stimulant and inhibitory effect of adrenaline in depolarized smooth muscle, J. Physiol. (London) *169*, 404.

Elliot, D.F., E.W. Horton and G.P. Lewis, 1960, Actions of pure bradykinin, J. Physiol. (London) *153*, 473.

Erdös, E.G., 1966, Hypotensive peptides: Bradykinin, kallidin and eledoisin, Advan. Pharmacol.*4*, 1.

Fishlock, D.J., 1966, Effect of bradykinin on the human isolated small and large intestine, Nature (London), *212*, 1533.

Gershon, M.D., 1967, Effect of tetrodotoxin on innervated smooth muscle preparations, Brit. J. Pharmacol. Chemother. *29*, 259.

Innes, I.R., H.W. Kosterlitz and J.A. Robinson, 1957, The effects of lowering bath temperature on the responses of the isolated guinea-pig ileum, J. Physiol. (London) *137*, 396.

Khairallah, P.A. and I.H. Page, 1963, Effects of bradykinin and angiotensin on smooth muscle, Ann. N.Y. Acad. Sci.*104*, 212.

Lewis, G.P. and E. Reit, 1965, The action of angiotensin and bradykinin on the superior cervical ganglion of the cat, J. Physiol. (London) *179*, 538.

Paton, W.D.M., 1955, The response of the guinea-pig ileum to electrical stimulation by coaxial electrodes, J. Physiol. (London) *127*, 40P.

Robison, G.A., R.W. Butcher and E.W. Sutherland, 1968, Cyclic AMP, Ann. Rev. Biochem. *37*, 149.

Rocha e Silva, M., 1970, In: Kinin hormones, Springfield, Ill.: C.C. Thomas Publishers.

Regoli, D. and J.R. Vane, 1964, A sensitive method for the assay of angiotensin, Brit.J. Pharmacol. Chemother. *23*, 351.

Sherman, W.T. and R.F. Gautieri, 1969, Cardiovascular and gastrointestinal effects of bradykinin and its potentiation by thiols in rats, J. Pharm. Sci. *58*, 971.

Schild, H.O., 1964, Pharmacology of smooth muscle, In: Proceedings of the Second International Pharmacology Meeting, Ed. E. Bülbring, p. 95, London: Pergamon Press.

Shibata, S., K. Hattori and D. Timmerman, 1970, Effect of cold storage on the response of guinea-pig taenia coli to certain catecholamines and other agents, European J. Pharmacol. *11*, 321.

Suzuki, T., A. Nishiyama and K. Okamura, 1964, The effects of barium ion on the resting and action potential of intestinal smooth muscle calls, Tokohu J. Exptl. Med. *82*, 87.

Takagi, K. and I. Takayanagi, 1962, Barium receptor on intestinal smooth muscle: mode of action of morphine on the papaverine-like antispasmodic action, Arch. Intern. Pharmacodyn. Ther. *135*, 223.

Trautschold, I., 1970, Assay methods in the kinin system, In: Handbook of Experimental Pharmacology, *25*, 52, ed. E.G. Erdös, New York: Springer-Verlag.

Wiegershausen, B., G. Stopp and M. Eichstädt, 1964, Zum Mechanismus der Bradykininwirkung am Meerschweinchendarm, Acta Biol. Med. German. *12*, 443.

Chapter 3

EFFECTS OF ADRENERGIC BLOCKERS ON THE RELAXATION OF THE GUINEA-PIG ILEUM BY BRADYKININ AND ADRENALINE

SUMMARY

Some α- and β-adrenergic blockers have been examined on the bradykinin-and adrenaline-induced relaxation of the acetylcholine contracted guinea-pig ileum. The α- adrenergic blocker piperoxan potentiated, while phentolamine reduced the bradykinin relaxation. Both reduced the acetylcholine contraction, but had no effect on the adrenaline relaxation. The bradykinin relaxation of the guinea-pig ileum was about ten times more sensitive to phentolamine than the rat duodenum.

The β-adrenergic blocker propranolol potentiated the bradykinin relaxation and reduced the acetylcholine contraction. Sotalol was in these respects less potent than propranolol. The adrenaline relaxation was partially blocked by propranolol, but almost completely by sotalol.

A combination of phentolamine and propranolol slightly potentiated the bradykinin relaxation, and partially blocked the adrenaline relaxation. The bradykinin relaxation is not due to a direct action on either the α- or β- adrenergic receptors of the guinea-pig ileum. The potentiation of the bradykinin relaxation is probably indirect via the reduction of the acetylcholine contraction. The action of phentolamine cannot be explained by this mechanism. Seemingly only β- adrenergic receptor activity mediates relaxation with adrenaline.

INTRODUCTION

Bradykinin is known to liberate adrenaline from the adrenal medulla (Lecompte et al., 1961; Feldberg and Lewis, 1964) and also to stimulate the sympathetic ganglia (Lewis and Reit, 1965). In the latter respect it resembles non-nicotinic ganglion stimulants (Lewis and Reit, 1966; Trendelenburg, 1966). Adrenergic blockade and adrenalectomy are known to potentiate the hypotensive (Rocha e Silva et al., 1960; Lloyd, 1962; Nakano, 1965) and the bronchoconstrictor (James, 1969) actions of bradykinin.

Some work has been devoted to examining these sympathomimetic effects of bradykinin on isolated smooth muscle preparations. The rat duodenum has been exclusively studied, since it may be relaxed with bradykinin at nanogram concentrations (Elliot et al., 1960) and this relaxation resembles sympathetic stimulation (Antonio, 1968). It is also known that stimulation of both α- and β-adrenergic receptors of intestinal smooth muscle by catecholamines results in relaxation (Brody and Diamond, 1967; Jenkinson and Morton, 1967). Some authors favour a sympathomimetic mechanism for the relaxation of the rat duodenum (Türker et al., 1964; Montgomery, 1968), but evidence incompatible with this hypothesis has also been presented (Antonio, 1968).

The finding that bradykinin can under suitable conditions relax the guinea-pig ileum (chapter 2) prompted an investigation into the possible sympathomimetic properties of bradykinin on this organ. The conditions that have been found suitable for the relaxation are the addition of bradykinin in the presence of contraction by low concentrations of acetylcholine and other agonists. In this study the hypothesis has been considered that bradykinin releases catecholamines from stores onto either the α- or β-adrenergic receptors, or has a direct action on the same receptors to mediate relaxation.

MATERIALS AND METHODS

Lengths of guinea-pig ileum, 2 cm, were prepared according to the previously described method (chapter 2). Using male albino rats (ca 200 g) T.N.O. strain Holland, pieces of duodenum were taken from the segment distal to the first 5 cm of the duodenum from the stomach. A 4-min cycle time was used with the guinea-pig ileum for the addition of acetylcholine and bradykinin or adrenaline, the acetylcholine being in contact with the ileum 1 min 40 sec and the bradykinin or adrenaline 40 sec before washing out. With the rat duodenum a 4-min cycle time was used for both bradykinin and adrenaline, the substances being in contact with the duodenum 1 min before washing out. The α- and β-adrenergic blockers were added 1 min before the addition of acetylcholine with the guinea-pig ileum, and with the rat duodenum 2 min before the addition of bradykinin. A 3-min cycle time was used

to examine the α- and β-adrenergic blockers on the acetylcholine contractions, the blockers being added 1 min before the addition of acetylcholine. The acetylcholine was in contact with the ileum 1 min before washing out. The height of the contraction was measured at the termination of the 1-min contact time.

Drugs used additional to those in chapter 2: phentolamine (Regitin R, CIBA-Geigy, Switzerland), piperoxan (May & Baker, England), propranolol (Inderal R, ICI, England), sotalol (MJ 1999, Mead Johnson, U.S.A.).

RESULTS

α-Adrenergic antagonists

Phentolamine, an imidazoline derivative and piperoxan, a benzodioxane derivative were chosen for examination as being typical competitive antagonists of α-adrenergic receptors. Fig. 1a shows the effect of piperoxan on the bradykinin and adrenaline relaxation. In the case of bradykinin a potentiation of the relaxation can be seen but with adrenaline there was neither potentiation nor blockade. At the same time phentolamine (fig. 1b) like piperoxan had no effect on the adrenaline relaxation, but unlike piperoxan the bradykinin relaxation was decreased. It can be observed in fig. 1 that with the adrenaline response there was a small but significant contraction immediately preceeding the maintained relaxation in the control responses. Both piperoxan and phentolamine blocked this small contraction, which recovered after washing out. Both α-adrenergic antagonists reduced the acetylcholine contraction height measured 1 min after addition of acetylcholine. The latter results suggested that there may be a correlation between the magnitude of the acetylcholine contraction and the bradykinin relaxation.

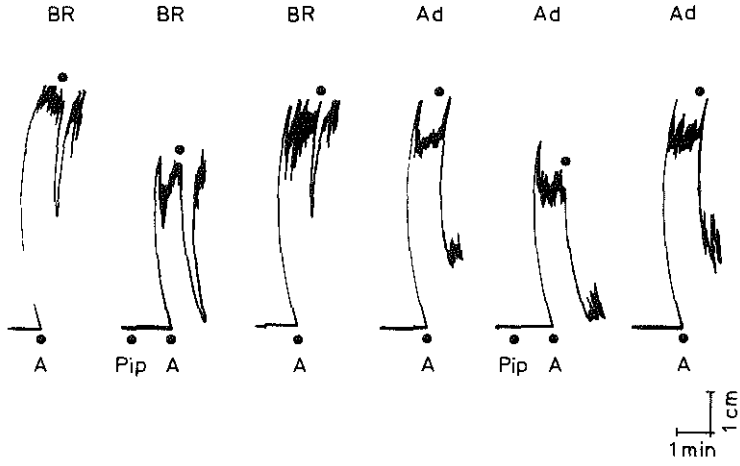

Fig. 1a

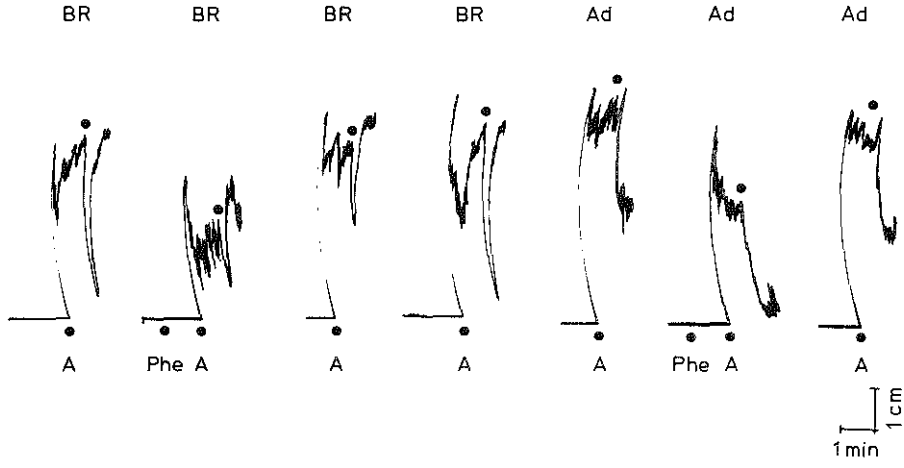

Fig. 1b

Fig. 1. The effect of piperoxan (fig. 1a, Pip, 4 µg/ml) and phentolamine (fig. 1b, Phe 1 µg/ml) on the bradykinin (BR, 2 ng/ml) and the adrenaline (Ad, 1 µg/ml) relaxation of the acetylcholine (A, 20 ng/ml) contracted guinea-pig ileum. Piperoxan or phentolamine was added 1 min before acetylcholine, and bradykinin or adrenaline 1 min after acetylcholine. Acetylcholine was in contact with the ileum 1 min 40 sec before washing out.

β-*Adrenergic antagonists*

The use of the two kinds of β-adrenergic blockers, propranolol and sotalol (MJ 1999), is justified on the grounds that sotalol has less marked local anaesthetic properties than propranolol (Somani et al., 1966). Fig. 2a shows that propranolol potentiated the bradykinin relaxation, while partially blocking the adrenaline relaxation. In fig. 2b can be seen the action of sotalol. Although the adrenaline

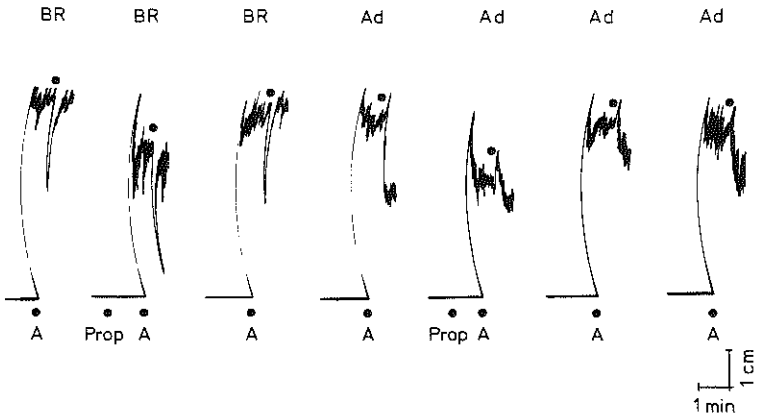

Fig. 2a

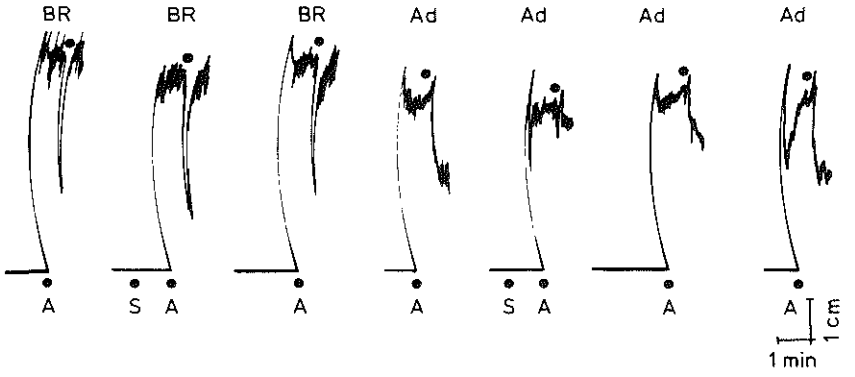

Fig. 2b

Fig. 2. The effect of propranolol (fig. 2a, Prop, 2 µg/ml) and sotalol (fig. 2b, S, 20 µg/ml) on the bradykinin (BR, 2 ng/ml) and the adrenaline (fig. 2a, Ad, 1 µg/ml; fig. 2b, 2 µg/ml) relaxation of the acetylcholine (A, 20 ng/ml) contracted guinea-pig ileum. Propranolol or sotalol was added 1 min before the addition of acetylcholine, and bradykinin or adrenaline 1 min after acetylcholine. Acetylcholine was in contact with the ileum 1 min 40 sec before washing out.

relaxation was almost entirely blocked, the bradykinin relaxation was hardly affected. The adrenaline contraction was not blocked by either of the β-adrenergic blockers. Although the acetylcholine spike heights were similar after propranolol, the contraction after 1 min was markedly depressed. Only a slight effect was seen on the acetylcholine contraction with sotalol. A quantitive summary of the results with α- and β-adrenergic blockers can be found in table 1.

Table 1

The effect of some α- and β-adrenergic blocking agents on the bradykinin and adrenaline relaxation of the acetylcholine contracted guinea-pig ileum.

Drug	Bradykinin[×] relaxation ± S.E.M. mm	Percentage		Adrenaline[××] relaxation ± S.E.M. mm	Percentage	
		Blockade	Potentiation		Blockade	Potentiation
Control*	31.0 ± 2.8 (6)**			31.2 ± 2.4 (6)		
Piperoxan 4 µg/ml	50.0 ± 4.2 (6)Δ	-	61.3	31.3 ± 2.4 (6)	-	0.3
Control	39.0 ± 0.8 (5)			36.8 ± 0.8 (5)		
Phentolamine 1 µg/ml	17.2 ± 0.5 (5)Δ	55.9	-	38.0 ± 1.7 (5)	-	3.3
Control	26.3 ± 1.4 (7)			24.6 ± 5.9 (5)		
Propranolol 2 µg/ml	39.6 ± 2.1 (7)Δ	-	50.6	7.2 ± 4.6 (5)Δ	70.7	-
Control	33.8 ± 3.7 (5)			23.6 ± 3.8 (5)		
Sotalol 20 µg/ml	34.7 ± 4.0 (5)	-	2.7	2.0 ± 1.3 (5)Δ	91.5	-
Control	19.0 ± 0.8 (4)			19.9 ± 2.2 (4)		
Phentolamine 2 µg/ml + propranolol 2 µg/ml	23.0 ± 0.9 (4)Δ	-	21.1	5.3 ± 0.6 (4)Δ	73.4	-

* Acetylcholine (20 ng/ml), except with phentolamine + propranolol (10 ng/ml), was added 1 min before bradykinin or adrenaline.

** Numbers of experiments in parentheses.

× Bradykinin, 2 ng/ml.

×× Adrenaline, 1 µg/ml, except with sotalol 2 µg/ml.

Δ $p \leq 0.05$ (Student's t-test).

α- and β-adrenergic blockers on the acetylcholine contraction

α- and β-adrenergic blockers were examined for their anti-acetylcholine effects on the guinea-pig ileum. The results are shown in table 2. From these the percentage of the blockade of the acetylcholine

Table 2

The influence of some α- and β-adrenergic blocking agents on the acetylcholine contraction of the guinea-pig ileum, and calculation of the expected bradykinin relaxation.

Drug	Acetylcholine contraction 20 ng/ml				Bradykinin relaxation 2 ng/ml		p*
	Control ± S.E.M. mm	Treated ± S.E.M. mm	% blockade	% maximum contraction on blockade ○	Expected △ ± S.E.M. mm	Observed ▲ ± S.E.M. mm	
Piperoxan 4 μg/ml	33.2 ± 1.5	20.5 ± 1.6	38.2 ± 2.1	38.9 ± 1.4 (10)**	36.1 ± 0.8	34.8	NS
Phentolamine 1 μg/ml	44.3 ± 0.9	23.9 ± 0.5	46.0 ± 2.1	34.0 ± 1.0 (10)	39.0 ± 0.6	9.5	0.0005
Propranolol 2 μg/ml	56.2 ± 1.0	42.0 ± 3.2	25.3 ± 1.8	47.1 ± 1.2 (9)	31.3 ± 0.6	32.5	NS
Sotalol 20 μg/ml	46.1 ± 1.2	44.6 ± 1.1	3.2 ± 3.7	61.0 ± 2.3 (10)	23.2 ± 1.4	22.2	NS

○ 20 ng/ml acetylcholine is taken to give 63% maximum contraction for these calculations.

△ Calculated on the basis of a linear regression between the percentage of maximum contraction with acetylcholine (x) and the relaxation of a standard dose of bradykinin (2 ng/ml) (y) for each value, then averaged: $y = -0.58x + 58.62$

▲ The observed relaxation with bradykinin (2 ng/ml) has been corrected assuming linearity between 1-4 ng/ml, and an average control value of 21.6 ± 1.2 (± S.E.M. mm, n = 11). Taken from the previously established dose-response relationship (chapter 2). See text for further details.

* Significant differences were determined by the x^2-test. (NS - Not significant)

** Number of experiments in parentheses.

contraction has been calculated at the particular blocker concentration used. The percentage maximum contraction on blockade was then calculated using the information that a 20 ng/ml bath solution of acetylcholine produces a 63 % maximum contraction of the ileum (chapter 2). For any percentage of maximum contraction there would be an expected relaxation with the standard dose of bradykinin (2 ng/ml). This expected bradykinin relaxation can be calculated from the regression equation of the percentage of maximum contraction with acetylcholine (x) and the relaxation (mm) of a standard dose of bradykinin (2 ng/ml) (y), where $y = -0.58x + 58.62$ (chapter 2). The relaxations with bradykinin actually observed were taken from table 1 and corrected so as

to lie on the common linear dose-response relationship between 1-4 ng/ml and giving a control response of 21.6 mm (2 ng/ml bradykinin) as shown in chapter 2. The control value corresponds to that found in the dose-response relationship of the bradykinin relaxation after acetylcholine (20 ng/ml). The expected and observed relaxations were then statistically examined by the Chi-squared test. It can be seen that in the case of propranolol, piperoxan and sotalol there is no difference between the relaxation that one would expect and that actually observed. With phentolamine the null hypothesis must be disregarded, there being a significant difference between the expected and observed results ($p = 0.0005$). Blockade of the bradykinin relaxation although observed to be 55.9 % with 1 µg/ml phentolamine is actually greater since phentolamine has been shown to block the acetylcholine contraction, thus giving higher theoretical control relaxations than those without phentolamine. This creates a greater difference between the theoretical control and the phentolamine treated experiments. The amount of blockade expected is 76.6 %.

Combination of α- and β-adrenergic blockers

Our experiments do not indicate participation of α-adrenergic receptors as mediators of the relaxation response of adrenaline on the guinea-pig ileum, even though it is commonly thought that both α- and β-adrenergic receptors contribute to the relaxation effect of catecholamines on intestinal smooth muscle (Jenkinson and Morton, 1967; Lum et al., 1966). However to achieve a complete blockade of the adrenaline response, and possibly block any sympathomimetic action of bradykinin resistant to α- and β-adrenergic blockers alone we have used a combination of phentolamine and propranolol. It can be seen in table 1 that the adrenaline relaxation was not completely blocked by the use of the blockers together and moreover no significantly greater degree of blockade was obtained than with propranolol alone. The bradykinin relaxation on the other hand was not diminished but slightly potentiated ($p = 0.025$). In table 1 there is an apparent large variation in the control responses to both bradykinin and adrenaline. The reason for this variation is the use of different recorder module sensitivities; however, during a series of experiments with a particular drug the sensitivity was constant.

Action of phentolamine on the rat duodenum

The sensitivities of the rat duodenum and the guinea-pig ileum were compared with regard to the effect of phentolamine on the bradykinin relaxation. This was undertaken since it had been previously reported that phentolamine was unable to decrease the relaxation of the rat duodenum (Antonio, 1968). In fig. 3 can be seen typical traces

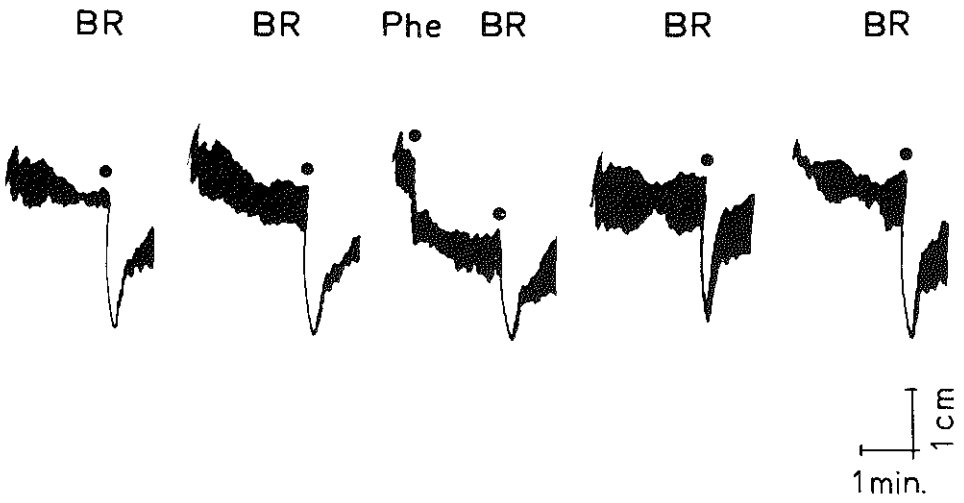

Fig. 3. The effect of phentolamine (Phe, 10 µg/ml) on the bradykinin (BR, 1 ng/ml) relaxation of the rat duodenum. Bradykinin was in contact with the ileum 1 min before washing out.

from the rat duodenum with bradykinin, and further some reduction after phentolamine. It should be noted that there was a decrease in the tonus of the duodenum after the addition of phentolamine. Dose-response relationships were constructed for both organs with phentolamine (table 3). It can be seen from table 3 that the bradykinin relaxation of the guinea-pig ileum is about 10 times more sensitive to the effect of phentolamine than the rat duodenum. The maximum measurable effect on the guinea-pig ileum by phentolamine was 67.2 % at 4 µg/ml, and with the rat duodenum 60.1 % at 20 µg/ml. It was not

Table 3

The effect of phentolamine on the bradykinin relaxation of the acetylcholine contracted guinea-pig ileum and the rat duodenum with normal tone.

Phentolamine µg/ml	GUINEA-PIG ILEUM Δ Bradykinin relaxation			RAT DUODENUM ○ Bradykinin relaxation		
	Control ± S.E.M. mm	Treated ± S.E.M. mm	% blockade	Control ± S.E.M. mm	Treated ± S.E.M. mm	% blockade
0.2	28.1 ± 4.5	27.0 ± 4.1	3.9 (4)**	-	-	-
0.4	33.8 ± 0.7	26.2 ± 0.9*	22.5 (5)	-	-	-
1.0	39.0 ± 0.8	17.2 ± 0.5*	55.9 (5)	16.8 ± 1.7	15.5 ± 1.7	7.7 (4)
2.0	17.6 ± 3.5	7.5 ± 1.9*	57.4 (5)	20.3 ± 0.3	18.8 ± 0.6	7.3 (4)
4.0	17.4 ± 4.7	5.7 ± 1.2*	67.2 (5)	20.2 ± 0.2	16.6 ± 0.5*	17.8 (5)
10.0	-	-	-	22.0 ± 1.2	14.2 ± 0.8*	34.5 (5)
20.0	-	-	-	21.8 ± 0.8	8.7 ± 0.6*	60.1 (6)

Δ Acetylcholine, 20 ng/ml, was added 1 min before bradykinin, 2 ng/ml.

○ Bradykinin was 1 ng/ml.

* $p \leq 0.05$ (Student's t-test).

** Numbers of experiments in parentheses.

possible to use higher concentrations of phentolamine on either of the organs than those shown because, with the ileum, the acetylcholine contraction was reduced even more than 46 % and with the duodenum the tonus was lowered which prevented measurement of the resulting bradykinin relaxation.

DISCUSSION

The sensitivity of the acetylcholine contracted guinea-pig ileum to relaxation by bradykinin is of the same order (1 ng/ml) as that of the rat duodenum or the contraction of the rat uterus. The relaxation of the guinea-pig ileum with adrenaline and noradrenaline is found to be not so rapid in onset as that with bradykinin, and a small contraction is observed immediately preceeding the relaxation. The relaxation with adrenaline is maintained unlike that with bradykinin which returns to the elevated base-line.

Although piperoxan and phentolamine are α-adrenergic blockers they have different actions on the bradykinin relaxation. Piperoxan is found to potentiate and phentolamine to prevent the bradykinin relaxation. Both compounds were without effect on the adrenaline relaxation, but blocked the contraction component of the adrenaline response. The foregoing data thus clearly show that the effect of the α-adrenergic blockers is dissimilar on responses to bradykinin and adrenaline respectively. It would appear that the bradykinin-induced relaxation is not mediated through a triggering of α-adrenergic receptors. The phentolamine-induced reduction in the relaxation response by bradykinin is at variance with the results reported for phentolamine and bradykinin on the rat duodenum (Antonio, 1968). This variance may be explained by the different doses used. The component of contraction of the adrenaline response is likely mediated via a stimulation of α-adrenergic receptors.

Neither of the β-adrenergic blockers prevented the bradykinin relaxation though propranolol was found to potentiate it. Both β-adrenergic blockers were found to antagonize the adrenaline relaxation, and at the concentrations used sotalol more effectively than propranolol. The contraction component of the adrenaline response is not blocked but slightly potentiated by the β-adrenergic blockers. The actions of the β-adrenergic blockers on the bradykinin and adrenaline responses are markedly different. It thus seems unlikely that the relaxation response by bradykinin is mediated by a stimulation of the β-adrenergic receptors. Conflicting results with β-adrenergic blockers on both the adrenaline and bradykinin responses have been found on the rat duodenum in the past (Türker et al., 1964; Antonio, 1968). It appears probable that the guinea-pig ileum has a different specificity towards adrenaline and bradykinin than the rat duodenum. In particular both α- and β-adrenergic receptors may play a role in the adrenaline relaxation of the rat duodenum, while for the guinea-pig ileum only β-adrenergic receptors. The contraction component of the adrenaline response on the guinea-pig ileum is likely mediated though a stimulation of the α-adrenergic receptors.

With the guinea-pig ileum either of the β-adrenergic blockers alone are as effective as a combination of phentolamine and propranolol. This provides further evidence supporting the previously discussed

proposal that the component of α-adrenergic receptor stimulation to mediate relaxation plays an insignificant role in this organ, but may be more important in the production of contraction responses. A combination of phentolamine and propranolol was found to have no effect on the bradykinin relaxation of the rat duodenum (Antonio, 1968), while the present results on the guinea-pig ileum demonstrate a small but significant potentiation of the bradykinin relaxation. This potentiation is smaller than that of propranolol alone, and is suggested to be the result of arithmetic addition of the two effects, potentiation by propranolol and reduction by phentolamine.

In chapter 2 it was shown that there was no tachyphylaxis of the bradykinin relaxation on the guinea-pig ileum. This was presented as argument against the release of mediators. The present results support the hypothesis that catecholamines are not being released onto the α- or β-adrenergic receptors. There is, however, evidence that could be used in support of catecholamine release by bradykinin in the rat intestines (Montgomery, 1968). He concludes that the predominance of either contraction or relaxation in the rat small intestines to bradykinin is related to the catecholamine concentration in the various regions; indirect relaxation occurring in those regions containing the highest amounts of catecholamines. The conclusion drawn from the latter results is in contradiction to those of the present on the guinea-pig ileum and those on the rat duodenum (Antonio, 1968). Although no clear cut explanation can be given for these discrepances, it is possible that if Montgomery used high concentrations of α- and β-adrenergic blockers (no concentrations were given in his communication) then the tone of the organ would be so reduced as to give the incorrect conditions for a relaxation with any substances. Other evidence presented involving reserpine does not necessarily implicate α- or β-adrenergic receptors.

Piperoxan, phentolamine and propranolol have been shown to markedly reduce, while sotalol only to slightly reduce the acetylcholine contraction. It has been previously demonstrated (chapter 2) that there exists a linear inverse relationship between either the acetylcholine concentration or the percentage of maximum contraction and the bradykinin relaxation of the guinea-pig ileum. In consequence a reduction in the acetylcholine contraction should result in an increase of the

bradykinin relaxation. In table 2 is shown the calculations necessary to measure an expected bradykinin relaxation as predicted from the regression equation, and further this is compared statistically using the Chi-squared test with the actual observed change in the bradykinin relaxation. Statistical analysis shows that there is no difference between the expected and observed bradykinin relaxations using piperoxan, propranolol and sotalol. It can be concluded from these results that the reduction of the acetylcholine contraction is probably the underlying cause of the potentiation of the bradykinin relaxation. In the case of sotalol there is little effect on the acetylcholine contraction or the bradykinin relaxation. In the latter respect there may be correlation with the local anaesthetic properties of the different blockers viz. propranolol and sotalol on the muscle cell membrane. The phentolamine results on the other hand indicate a larger reduction than that actually observed. This might explain why only approximately 60 % reduction can be achieved in practice. It seems that under the conditions of these experiments one must consider not only the influence of drugs acting directly on the bradykinin relaxation, but also an indirect influence because of their anti-cholinergic activities. Indeed a similar situation may exist with the rat duodenum and generally for smooth muscle, where any changes in the tonus of the organ might have an influence on the measurement of the bradykinin relaxation. These possibilities are further discussed in chapter 4.

REFERENCES

Antonio, A., 1968, The relaxing effect of bradykinin on intestinal smooth muscle, Brit. J. Pharmacol. Chemother. *32*, 78.

Brody, T.M. and J. Diamond, 1967, Blockade of the biochemical correlates of contraction and relaxation in uterine and intestinal smooth muscle, Ann. N.Y. Acad. Sci. *139*, 772.

Elliot, D.F., E.W. Horton and G.P. Lewis, 1960, Actions of pure bradykinin, J. Physiol. (London) *153*, 473.

Feldberg, W. and G.P. Lewis, 1964, The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin, J. Physiol. (London) *171*, 98.

James, G.W.L., 1969, The use of the *in vivo* trachea preparation of the guinea-pig to assess drug action on the lung, J. Pharm. Pharmacol. *21*, 379.

Jenkinson, D.H. and I.K.M. Morton, 1967, The role of α- and β-adrenergic receptors in some actions of catecholamines on intestinal smooth muscle, J. Physiol. (London) *188*, 387.

Lecompte, J., J. Troquet and A. Dresse, 1961, Stimulation médullo-surrénalienne par la bradykinine, Arch. Intern. Physiol. Biochem. *69*, 89.

Lewis, G.P. and E. Reit, 1965, The action of angiotensin and bradykinin on the superior cervical ganglion of the cat, J. Physiol. (London) *179*, 538.

Lewis, G.P. and E. Reit, 1966, Further studies on the actions of peptides on the superior cervical ganglion and suprarenal medulla, Brit. J. Pharmacol. Chemother. *26*, 444.

Lloyd, S., 1962, Vascular responses of the rat to bradykinin, Brit. J. Pharmacol. Chemother. *19*, 503.

Lum, B.K.B., M.H. Kermani and R.D. Heilman, 1966, Intestinal relaxation produced by sympathomimetic amines in the isolated rabbit jejunum:selective inhibition by adrenergic blocking agents and by cold storage, J. Pharmacol. exp. Therap. *154*, 463.

Montgomery, E.H., 1968, The response of the rat duodenum to bradykinin, Proc. West. Pharm. Soc. *11*, 51.

Nakano, J., 1965, Effects of synthetic bradykinin on the cardiovascular system, Arch. Intern. Pharmacodyn. Ther. *157*, 1.

Rocha e Silva, M., A.P. Corrado and A.O. Ramos, 1960, Potentiation of duration of the vasodilator effect of bradykinin by sympatholytic drugs and by reserpine, J. Pharmacol. exp. Therap. *128*, 217.

Somani, P., J.G. Fleming, G.K. Chan and B.K.B. Lum, 1966, Antagonism of epinephrine-induced cardiac arrhythmias by 4- (2-isopropylamino-1-hydroxyethyl)-methanesulfonanilide (MJ 1999), J. Pharmacol. exp. Therap. *151*, 32.

Trendelenburg, U., 1966, Observations on the ganglion-stimulating actions of angiotensin and bradykinin, J. Pharmacol. exp. Therap. *154*, 418.

Türker, K., B.K. Kiran and S. Kaymakçalan, 1964, The effects of synthetic bradykinin on intestinal motility in different laboratory animals and its relation to catecholamines, Arch. Intern. Pharmacodyn. Ther. *151*, 260.

Chapter 4

MECHANISM OF THE INHIBITORY ACTIONS OF SOME SPASMOGENS ON THE BRADYKININ-INDUCED RELAXATION OF THE GUINEA-PIG ILEUM

SUMMARY

The bradykinin relaxation of the acetylcholine contracted guinea-pig ileum is decreased by increasing the concentration of acetylcholine. This finding prompted an investigation to determine whether other spasmogens can reduce the bradykinin relaxation; and the mechanisms involved.

Linear inverse relationships were found between both the percentage of maximum contraction and concentrations of acetylcholine, histamine and eledoisin, and the bradykinin relaxation. Acetylcholine showed a steeper regression equation slope than histamine or eledoisin. No relationship existed for angiotensin, though low non-spasmogenic doses in the presence of the acetylcholine-contracted guinea-pig ileum reduced the bradykinin relaxation. Morphine and atropine reduced the angiotensin contraction and potentiated the bradykinin relaxation in its presence. Only atropine reduced the acetylcholine contraction and potentiated the bradykinin relaxation in its presence. Neither morphine nor atropine had any effect on the bradykinin reduction of its own responses.

The magnitude of the bradykinin relaxation on the guinea-pig ileum is dependent on the manner of stimulation, rather than on the state of contraction, and also is sensitive to regulation by agents that have their actions on the parasympathetic system. Angiotensin reduces the bradykinin relaxation by a mechanism involving its indirect parasympathomimetic effects. In contrast the bradykinin reduction of its own relaxation is not via a parasympathetic mechanism, but is probably a direct action.

INTRODUCTION

Bradykinin is known to contract smooth muscle preparations such as the isolated rat uterus, the guinea-pig ileum and the rat stomach.

However, certain intestinal smooth muscle preparations such as those of the rat duodenum and the hen rectal caecum are relaxed by bradykinin (Rocha e Silva, 1970). In chapter 2 has been shown that bradykinin can relax the isolated guinea-pig ileum in the presence of contractions by acetylcholine and other spasmogens, and further in chapter 3, the involvement of catecholamines has been ruled out. Increasing the concentration or percentage of maximum contraction of the guinea-pig ileum with acetylcholine decreased the bradykinin relaxation in a linear manner. The present work was designed to investigate whether other spasmogens can reduce the bradykinin relaxation; and, the mechanisms involved.

MATERIALS AND METHODS

The isolated guinea-pig ileum preparations were prepared according to the method described in chapter 2. In the dose-response experiments used for the calculation of the percentage of maximum contraction, all responses were measured without alteration of the base-line. A 3-min cycle time was used to measure the dose-response characteristics of the different spasmogens. A 4-min cycle time was used for the addition of the spasmogens and bradykinin, the spasmogens being in contact with the ileum 1 min 40 sec, and the bradykinin 40 sec before washing out. The sensitivity of the recording module was constant throughout the experiments, however the base-line was adjusted by vertical displacement of the transducer where necessary, thus allowing the changes in the length of the ileum to be measured within the preset scale and sensitivity of the recorder. The maximum contraction with acetylcholine can be estimated to be about 125 mm.

In the experiments using low concentrations of angiotensin a 4-min cycle time was used; the angiotensin was added 1 min before a standard dose of acetylcholine, and without washing out bradykinin was added 1 min thereafter. A 3-min cycle time was used with the DMPP contractions, morphine being added 1 min before the DMPP. With DMPP the maximum contraction height attained was measured, while with angiotensin and acetylcholine the contraction height at the termination of 1 min contact time.

In the experiments examining the effect of morphine on the acetyl-

choline contraction and bradykinin relaxation, a 4-min cycle time was used, while an 8-min cycle time was used for the angiotensin/bradykinin relaxation experiments. An 8-min cycle time was necessary since angiotensin was difficult to completely wash out and tended to be tachyphylactic in a 4-min cycle time. Morphine was added 1 min before the spasmogen. A similar protocol was used for atropine, replacing morphine.

The bradykinin reduction of its own responses required the use of a 5-min cycle time, bradykinin being added twice at a 1 min interval after acetylcholine or histamine. The second bradykinin addition was washed out after a 30 sec contact time. Morphine or atropine was added 1 min before the spasmogens as required.

Drugs used in addition to those in chapter 2: atropine sulphate (Merck, Germany), 1,1-dimethyl-4-phenylpiperazinium iodide(DMPP) (Aldrich-Europe, Belgium), morphine hydrochloride (Brocades-ACF, Holland).

RESULTS

Various spasmogens on the bradykinin relaxation

Histamine, eledoisin and angiotensin were substituted for acetylcholine (see also chapter 2). Dose-response relationships for the increase in the concentration and contraction produced by the spasmogens were constructed. The maximum contraction heights were measured at the termination of 1 min contact time and all lower contractions expressed as the percentage of the maximum contraction of each spasmogen. The relaxations produced by a standard dose of bradykinin, 2 ng/ml, were measured after the different concentrations of spasmogens, some of which exceeded the concentrations required to produce the maximum contraction. The correlation coefficients were calculated for each of the spasmogens and the subsequent bradykinin relaxations. Further, when correlation was found, the regression equations for the various relationships were determined. The regression lines were calculated by the method of least squares, from which the standard deviations of the regression coefficients and the standard error of the estimate (scatter) were calculated (Documenta Geigy). These results are summarized in

table 1. Here is shown the magnitudes of the bradykinin relaxations after increasing concentrations of the spasmogens, and further the regression equations for the increase in the percentage of maximum contraction and the bradykinin relaxation. The percentages of maximum contraction are not shown in table 1, however, in table 2 the bradykinin relaxations are compared at identical percentages of maximum contraction (25, 50, 75, 100 %) for each spasmogen. The bradykinin relaxations were calculated by substitution of the fixed percentage of maximum contraction values into the respective regression equations.

Table 1

The influence of increasing doses of various contracting substances on the bradykinin relaxation of the isolated guinea-pig ileum.

Dose spasmogen ng/ml	Bradykinin relaxation ± SEM (mm) (2 ng/ml) Acetylcholine	Histamine	Eledoisin	Angiotensin
4	-	-	-	14.5 ± 0.7 (8)
10	43.0 ± 0.7 (6)**	-	42.1 ± 1.2 (6)	14.5 ± 1.0 (8)
20	21.6 ± 1.2 (11)	44.0 ± 0.4 (8)	39.6 ± 1.8 (6)	14.3 ± 1.2 (8)
40	8.8 ± 0.6 (5)	41.9 ± 0.6 (8)	37.0 ± 1.9 (4)	*14.9 ± 1.2 (8)
100	*1.9 ± 0.2 (5)	39.9 ± 0.8 (8)	*34.7 ± 0.9 (4)	15.0 ± 1.3 (8)
200	1.0 ± 0.0 (5)	*34.3 ± 1.2 (8)	27.2 ± 2.1 (4)	-

Derived mathematical relationships between percentage of maximum contraction of the spasmogens and the bradykinin relaxation

Correlation coefficient (r) $^{\Delta}$	- 0.99	- 0.93	- 0.63	no correlation
Regression coefficients $^{\Delta}$ a ± S.D.	58.62 ± 0.15°	48.88 ± 0.11$^{\blacktriangle}$	59.64 ± 0.66°	14.63 ± 0.47 $^{\blacktriangle\circ}$
b	- 0.58 ± 0.017°	- 0.13 ± 0.012$^{\blacktriangle}$	- 0.24 ± 0.070$^{\blacktriangle}$	0
Standard error of estimate	± 2.41	± 1.61	± 3.41	-

* Maximum contraction of spasmogen.

** Numbers of experiments in parentheses.

$^{\Delta}$ Correlation and regression coefficients based on concentrations of spasmogen which do not exceed those giving maximum contraction; generally $y = bx + a$, where y = bradykinin relaxation (mm), x = percentage of maximum contraction. (Regression coefficient a with angiotensin, ± S.E.M.).

$^{\blacktriangle}$ $p < 0.0005$ (acetylcholine), other pairs $p > 0.05$ (t-test for regression coefficients)

$^{\circ}$ $p < 0.0005$ (histamine), other pairs $p > 0.05$ (t-test for regression coefficients).

It can be seen from table 1 that the bradykinin relaxation diminishes with increasing doses of acetylcholine, while for eledoisin and histamine even high concentrations diminish the relaxation considerably less. With angiotensin there is no correlation between increasing concentrations or the percentage of maximum contraction and the bradykinin relaxation. The regression slopes for histamine and eledoisin were significantly different from that of acetylcholine, though only the regression constant for histamine was significantly different from that of acetylcholine, when the acetylcholine parameters are fixed. The regression slope for acetylcholine was significantly different from that of histamine, while the regression constants for acetylcholine and eledoisin were significantly different from that of histamine, when the histamine parameters are fixed. The constant for angiotensin was significantly different from that of acetylcholine and histamine. The statistical significance between the various parameters was calculated using the Student's t-test for regression coefficients (Documenta Geigy). In table 2 can be seen that at 25 % maximum contraction

Table 2

The influence of increasing the percentage of maximum contraction of the guinea-pig ileum by some spasmogens on the bradykinin relaxation.

Percentage of maximum contraction	BRADYKININ (2 ng/ml) RELAXATIONS* IN THE PRESENCE OF			
	Acetylcholine	Histamine	Eledoisin	Angiotensin
25	44.1	45.6	53.3	14.5
50	29.6	42.5	47.3	14.5
75	15.1	39.0	41.3	14.5
100	0.6	35.8	35.3	14.5

* The relaxations are calculated by substitution of the fixed percentage of maximum contraction parameter into the respective regression equations of table 1.

with acetylcholine and histamine, bradykinin produces very similar relaxations. After eledoisin the bradykinin relaxation is slightly higher, while after angiotensin it is very much lower. The results

indicate that increasing the percentage of maximum contraction with acetylcholine reduces the magnitude of the bradykinin relaxation greatest followed by eledoisin then histamine. Within the range of concentrations for angiotensin, as obtained when identical percentages of maximum contraction were considered no decrease in the bradykinin relaxation with increasing percentages of maximum contraction was observed. Not only was there a complete lack of dose-dependency between the angiotensin-induced contraction and the reduction of the bradykinin relaxation, but the relaxation was considerably smaller at 25 % maximum contraction than the other spasmogens.

Low doses of angiotensin on the bradykinin relaxation

To determine whether there existed a dose-response relationship at any concentration of angiotensin, and the bradykinin relaxation we examined the effect of lower concentrations of angiotensin (as compare to those in table 1), insufficient to produce a contraction, on the bradykinin relaxation of the acetylcholine contracted guinea-pig ileum. The results of these experiments are shown in table 3. It is observed

Table 3

The influence of low concentrations of angiotensin on the bradykinin relaxation of the acetylcholine contracted guinea-pig ileum.

Dose of angiotensin Δ ng/ml	BRADYKININ RELAXATION ± S.E.M. (mm) Control ○	Angiotensin treated	Percentage reduction in bradykinin relaxation
0.1	20.9 ± 0.5	20.6 ± 0.8 (5)**	-
0.2	24.3 ± 1.4	24.3 ± 1.4 (8)	-
0.4	25.3 ± 0.8	22.6 ± 0.8 (8)*	10.3
1.0	25.1 ± 0.6	19.5 ± 0.5 (9)*	22.0
2.0	24.5 ± 0.9	16.3 ± 1.4 (9)*	33.0
4.0	28.8 ± 0.6	22.3 ± 0.9 (8)*	22.7
10.0	25.5 ± 0.7	19.1 ± 1.1 (4)*	24.8

Δ Angiotensin was added 1 min before acetylcholine, 20 ng/ml.

○ Bradykinin, 2 ng/ml.

* $p \leq 0.01$ (Student's t-test).

** Numbers of experiments in parentheses.

that there is a reduction of the bradykinin relaxation between 0.2 - 2.0 ng/ml angiotensin. At doses of angiotensin 2 ng/ml and higher there was no further reduction of the bradykinin relaxation, even though the guinea-pig ileum could be seen to be slightly contracted at these concentrations.

Morphine on the bradykinin relaxation

We have examined morphine, 10 ng/ml, on the contraction of the guinea-pig ileum by acetylcholine and angiotensin, and further on the bradykinin relaxation in their presence. The concentration of morphine to be used, 10 ng/ml, was found to produce 77.4 % blockade of DMPP (1µg/ml) contractions. Control responses were 32.2 ± 0.8 (± S.E.M., mm) and morphine treated 7.3 ± 0.9 (± S.E.M., mm, 5 experiments).

Table 4

The effect of morphine on the angiotensin and acetylcholine contraction of the guinea-pig ileum, and on the bradykinin relaxation in their presence.

Spasmogen	Spasmogen contraction ± S.E.M. (mm)		Bradykinin relaxation° ± S.E.M. (mm)	
	Control	Morphine treated Δ	Control	Morphine treated
Angiotensin 10 ng/ml	53.1 ± 1.2	27.8 ± 1.7*(8)**	20.4 ± 0.7	30.6 ± 1.1*(17)
Acetylcholine 20 ng/ml	52.4 ± 1.0	52.7 ± 0.9 (8)	32.9 ± 1.3	34.1 ± 2.2 (9)

Δ Morphine was 10 ng/ml

o Bradykinin was 2 ng/ml

* $p > 0.0005$ (student's t-test)

** numbers of experiments in parentheses

In table 4 can be seen a summary of the results with morphine. A significant reduction of the angiotensin contraction was observed, though there was no effect on the acetylcholine contraction. Further

can be seen a significant potentiation of the bradykinin relaxation in the presence of angiotensin, though there is no effect in the presence of acetylcholine.

Atropine on the bradykinin relaxation

In a manner similar to the experiments with morphine was examined the effect of atropine on the acetylcholine and angiotensin contraction and on the bradykinin relaxation in their presence. The concentration of atropine to be used (1 ng/ml) was found to produce 45.4 % blockade of the DMPP (1µg/ml) contractions. Control responses were 26.0 ± 1.0 (± S.E.M., mm) and atropine treated 14.2 ± 1.3 (± S.E.M., mm, 5 experiments).

At this atropine concentration, it was found that the acetylcholine contraction was blocked to such a large extent that it was impossible to measure the bradykinin relaxation. In consequence atropine was used at 0.5 ng/ml for acetylcholine, while 1 ng/ml for angiotensin. The results of these experiments are summarized in table 5.

Table 5

The effect of atropine on the angiotensin and acetylcholine contraction of the guinea-pig ileum, and on the bradykinin relaxation in their presence.

Spasmogen	Spasmogen contraction ± S.E.M. (mm)		Bradykinin relaxation° ± S.E.M. (mm)	
	Control	Atropine treated △	Control	Atropine treated
Angiotensin 20 ng/ml	50.2 ± 1.3	41.0 ± 1.6*(6)**	14.2 ± 1.4	20.1 ± 1.5*(6)
Acetylcholine 20 ng/ml	55.4 ± 0.6	35.5 ± 0.6*(8)	21.2 ± 0.7	34.2 ± 0.7*(7)

△ Atropine was 1.0 ng/ml with angiotensin, 0.5 ng/ml with acetylcholine

o Bradykinin was 2 ng/ml

* $p \geqslant 0.025$ (student's t-test)

** Numbers of experiments in parentheses

A significant reduction of the angiotensin contraction was observed, and also a significant potentiation of the bradykinin relaxation. The acetylcholine contraction is partially blocked by atropine, and at the same time the bradykinin relaxation is potentiated. The blockade of the acetylcholine contraction was greater than that of angiotensin, despite the fact that with angiotensin the atropine concentration was twice that with acetylcholine. With acetylcholine in the presence of atropine a theoretical bradykinin relaxation can be calculated from the acetylcholine-bradykinin regression equation of table 1. This relaxation was 35.3 $\pm$ 0.4 ($\pm$ S.E.M., mm, 8 experiments). Statistical examination using the Chi squared test showed no significant difference between the theoretical relaxation and the observed relaxation in table 5.

Morphine and atropine on the bradykinin reduction of its own response

The effect of morphine, 10 ng/ml, and atropine, 1 ng/ml, has been examined on the bradykinin, 2 ng/ml, reduction of its own response with the acetylcholine, 20 ng/ml, contracted guinea-pig ileum for morphine (10 experiments), and the histamine, 40 ng/ml, contracted guinea-pig ileum for atropine (5 experiments). In fig. 1 can be seen traces sho-

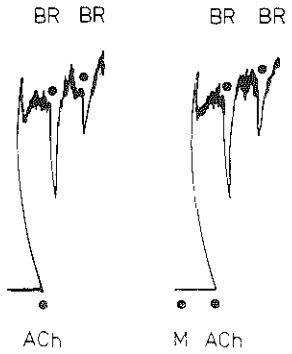

Fig. 1. The lack of effect of morphine (M, 10 ng/ml) on the bradykinin (BR, 2 ng/ml) reduction of its own relaxation response on the acetylcholine (ACh, 20 ng/ml) contracted guinea-pig ileum. Morphine was added 1 min before ACh, and bradykinin, added in two cumulative doses at a 1 min interval, 1 min after ACh.

wing the lack of effect of morphine on the bradykinin reduction of its own response. It is also observed that morphine has no effect on the acetylcholine contraction nor on the bradykinin relaxation (see also table 4). In a similar manner atropine did not alter the bradykinin reduction of its own response on the histamine contracted guinea-pig ileum, nor affect the histamine contraction or the bradykinin relaxation.

DISCUSSION

In chapter 2 it was demonstrated that there is a linear inverse relationship between variation in the percentage of maximum contraction of the guinea-pig ileum using acetylcholine and the bradykinin relaxation in the presence of acetylcholine. It thus appeared that the magnitude of the bradykinin relaxation was dependent on the extent of contraction of the ileum by acetylcholine. It was also reported that challenge of the ileum by some other spasmogenic substances provides the correct conditions for the bradykinin relaxation. The present work shows that increasing the concentrations of spasmogens such as histamine and eledoisin, does not seem to decrease the bradykinin relaxation as much as with acetylcholine. This is observed from the slopes of the regression equations, where acetylcholine is - 0.58, histamine - 0.13 and eledoisin - 0.24. In the case of angiotensin there is no correlation between increasing doses and the bradykinin relaxation. The various effects of the spasmogens on the bradykinin relaxation almost certainly reflects the difference in the mechanism of contraction of the ileum. It would appear that the fact that the state of contraction of the ileum can be changed is not sufficient criterion to expect a changed bradykinin relaxation. This can be further borne out, since when concentrations of the spasmogens were used exceeding those giving 100 % maximum contraction i.e. with acetylcholine and eledoisin, the relaxations with bradykinin were further decreased. It is likely that further increases in the concentrations of the spasmogens would decrease the bradykinin relaxation even further. Even the fact that the ileum is in a state of contraction is not sufficient criterion to predict a bradykinin relaxation. This conclusion was derived from earlier

experiments (chapter 2) involving contraction of the ileum with depolarizing concentrations of potassium or barium chloride, none of which provided the conditions suitable for relaxation by bradykinin. It appears possible that there is at least a partial common pathway for the action on the bradykinin relaxation by the spasmogens, but there are differences in degree of stimulation and/or saturation of the mechanism.

The larger reduction of the bradykinin relaxation in the presence of angiotensin as compared to that of histamine and eledoisin might be ascribed to the fact that angiotensin can exert an action on the guinea-pig ileum via a stimulation of the parasympathetic ganglia, with the result of the release of acetylcholine from the post-ganglionic nerve endings (Khairallah and Page, 1961, 1963; Robertson and Rubin, 1962; Godfraind et al., 1966). On the other hand angiotensin may be reducing the bradykinin relaxation by a mechanism similar to that by which bradykinin reduces its own relaxing effect, as shown in chapter 2.

To investigate the precise mechanisms involved the effects of morphine and atropine have been examined on the bradykinin relaxation in the presence of contraction by acetylcholine and angiotensin, and further in the presence of bradykinin. Morphine is known to decrease the amount of acetylcholine released from the pre- and postganglionic fibres (Paton, 1957; Pelikan, 1960). Acetylcholine contractions of the guinea-pig ileum were resistant to concentrations of morphine that antagonized DMPP-induced contractions. DMPP is a recognized ganglionic stimulant (Chen et al., 1951). Angiotensin contractions were antagonized by morphine, in agreement with Khairallah and Page (1961). The bradykinin relaxation in the presence of angiotensin, but not that in the presence of acetylcholine, was potentiated by morphine. These results strongly suggest that angiotensin by its stimulatory action on the parasympathetic ganglia attenuates the relaxing properties of bradykinin on the guinea-pig ileum. It is unlikely that exogenous acetylcholine exerts its action on bradykinin via a mechanism involving ganglionic stimulation, since morphine had no effect on the bradykinin relaxation in the presence of acetylcholine.

Atropine antagonized DMPP, acetylcholine and angiotensin-induced contractions of the guinea-pig ileum, though a somewhat lower concentration of atropine was required for antagonism of the acetylcholine

contraction. In the presence of acetylcholine and angiotensin the bradykinin relaxation could be seen to be potentiated. With acetylcholine the magnitude of the bradykinin relaxation as predicted from the regression equation was not significantly different from that observed. The results with atropine suggested a direct involvement of the muscarinic acetylcholine receptors, rather than some other direct or indirect effect of acetylcholine. By analogy the potentiation in the presence of angiotensin and atropine can be ascribed to the antagonistic activity of atropine on the muscarinic acetylcholine receptors. It is understandable that ganglionic stimulants would be slightly more resistant to the action of atropine since the acetylcholine is released in closer proximity to the receptors. It is difficult to equate that changes in spasmogenic concentrations of angiotensin produced no alteration in the bradykinin relaxation, while atropine reduced the angiotensin contraction and potentiated the bradykinin relaxation in its presence. One might speculate that the cholinergic component of the angiotensin action, which has its effects on the bradykinin relaxation, is saturated at low, non-spasmogenic concentrations, and this activity is separable from its indirect spasmogenic effect, but both activities are blocked by atropine and morphine.

It has been suggested that for the bradykinin contraction of the isolated guinea-pig ileum there exists an indirect mechanism via the release of acetylcholine (Wiegershausen et al., 1964), though evidence has been presented implicating only direct mechanisms (Day and Vane, 1963; Khairallah and Page, 1963; Gershon, 1967). The former indirect mechanism was the result of the finding that atropine and morphine reduced the contraction speed of the ileum to bradykinin, while eserine increased the contraction speed. Whereas the bradykinin contraction height of the guinea-pig ileum was unaffected by morphine or atropine (Walaszek et al., 1963; Wiegershausen et al., 1964), the inhibitory phase (probably equivalent to a relaxation on the ileum with raised tone) on the rabbit intestine was blocked by morphine, but not by atropine (Bauer et al., 1966). However, in the rat intestine the inhibitory effect was not blocked by either morphine or atropine (Bauer et al., 1966). One might conclude that for the guinea-pig ileum there is some form of cholinergic stimulation by bradykinin, but this does not contribute to the magnitude of the bradykinin contraction. It was

considered that the effects of atropine and morphine, and eserine on the contraction speed of the guinea-pig ileum may be a reflection, not of an action on the bradykinin contraction, but on the bradykinin relaxation. The rationale of this suggestion would be that the magnitude of the bradykinin relaxation, which occurs in the so called lag phase is regulated by the inherent parasympathomimetic properties of bradykinin. In an attempt to substantiate this hypothesis the effects of morphine and atropine on the bradykinin reduction of its own relaxing effect have been examined. Under no circumstances did atropine or morphine relieve the regulating influence of bradykinin on its own relaxation response, in contrast to the action of angiotensin on bradykinin; and further, morphine was not observed to affect the bradykinin relaxation in the presence of acetylcholine. It must be concluded that the bradykinin reduction of its own relaxing effect, in contrast to the reduction of the bradykinin relaxation by angiotensin, is not mediated via an action on the cholinergic innervation of the guinea-pig ileum, nor is the bradykinin relaxation a consequence of cholinergic stimulation. These conclusions are not in contradiction to the concept of regulation in the magnitude of the bradykinin relaxation by cholinergic stimulation.

Recently it has been suggested that potentiation of the bradykinin contraction by cysteine on the isolated guinea-pig ileum with normal tone is indirect via facilitation of acetylcholine release from nerve endings (Potter and Walaszek, 1972). It would be reasonable to suppose that other substances and manipulations producing these indirect effects would also potentiate the bradykinin contraction. Although exogenous acetylcholine was not able to potentiate the bradykinin relaxation (chapter 2), this does not rule out a neurogenic mechanism for the ileum with normal tone. Consideration of these results suggested that the magnitude of the components of the bradykinin response of the guinea-pig ileum may be regulated by the parasympathetic tone of the organ. If this is the case then the smaller regression slopes with histamine and eledoisin, as compared to acetylcholine, would be explained if a cholinergic mechanism was required to effect substantial changes in the bradykinin relaxation.

REFERENCES

Bauer, G., E. Ziegler and H. Konzett, 1966, Zur Hemmwirkung von Kininen am isolierten Darmpräparaten, Naunyn-Schmiedebergs Arch. exp. Path. u. Pharmak. *254*, 235.

Chen, G., R. Portman and A. Wickel, 1951, Pharmacology of 1,1-dimethyl-4-phenylpiperazinium iodide, a ganglion stimulating agent, J. Pharmacol. exp. Ther. *103*, 330.

Day, M. and J.R. Vane, 1963, An analysis of the direct and indirect actions of drugs on the isolated guinea-pig ileum, Brit. J. Pharmacol. Chemother. *20*, 150.

Gershon, M.D., 1967, Effect of tetrodotoxin on innervated smooth muscle preparations, Brit. J. Pharmacol. Chemother. *29*, 259.

Godfraind, T., A. Kaba and P. Polsten, 1966, Specific antagonism to the direct and indirect action of angiotensin on isolated guinea-pig ileum, Brit. J. Pharmacol. Chemother. *28*, 93.

Khairallah, P.A. and I.H. Page, 1961, Mechanism of action of angiotensin and bradykinin on smooth muscle *in situ*, Amer. J. Physiol. *200*, 51.

Khairallah, P.A. and I.H. Page, 1963, Effects of angiotensin and bradykinin on smooth muscle, Ann. N.Y. Acad. Sci. *104*, 212.

Paton, W.D.M., 1957, The action of morphine and related substances on contraction and acetylcholine output of coaxially stimulated guinea-pig ileum, Brit. J. Pharmacol. Chemother. *12*, 119.

Pelikan, E.W., 1960, The mechanism of ganglionic blockade produced by nicotine, Ann. N.Y. Acad. Sci. *90*, 52.

Potter, D.E. and E.J. Walaszek, 1972, Potentiation of the bradykinin response by cysteine: Mechanism of action, Arch. Intern. Pharmacodyn. Ther. *197*, 338.

Robertson, P.A. and D. Rubin, 1962, Stimulation of intestinal nervous elements by angiotensin, Brit. J. Pharmacol. Chemother. *19*, 5.

Walaszek, E.J., C.G. Huggins and C.M. Smith, 1963, Drugs that modify the actions of pharmacologically active polypeptides, Ann. N.Y. Acad. Sci. *104*, 281.

Wiegershausen, B., G. Stopp and M. Eichstädt, 1964, Zum Mechanismus der Bradykininwirkung am Meerschweinschendarm, Acta Biol. Med. German. *12*, 443.

Chapter 5

EFFECT OF PHENTOLAMINE ON THE BRADYKININ RELAXATION OF THE GUINEA-PIG ILEUM CONTRACTED BY VARIOUS SPASMOGENS

SUMMARY

The phentolamine reduction of the bradykinin relaxation on the isolated guinea-pig ileum was studied in the presence of various spasmogens. Phentolamine reduced the bradykinin relaxation most potently after acetylcholine, followed by angiotensin, then histamine and eledoisin. Phentolamine invariably reduced the bradykinin relaxation, but the amount of reduction was dependent on the initial spasmogen. In this respect phentolamine reduced the histamine contractions greatest followed by acetylcholine and angiotensin. Eledoisin contractions were not affected by phentolamine. It was concluded that phentolamine does not have a completely aspecific spasmolytic action.

INTRODUCTION

It was observed in chapter 3 that piperoxan and propranolol potentiated the bradykinin relaxation, while phentolamine, though not in the role of an α-adrenergic blocker, could be seen to reduce the relaxation. Potentiation of the bradykinin relaxation could be explained by a piperoxan or propranolol induced reduction of the initial acetylcholine contraction; since, a linear inverse relationship between the increase in the acetylcholine concentration and the relaxation produced by a constant dose of bradykinin had been previously established (chapter 2). The present experiments were designed to investigate the influence of various spasmogens on the reduction of the bradykinin relaxation by phentolamine.

MATERIALS AND METHODS

The isolated guinea-pig ileum preparations were set-up according to a previously described method (chapter 2). The sensitivity of the

recording module was constant throughout the experiments, however the base-line before measurement of contractions was adjusted by vertical displacement of the transducer where necessary, thus allowing the changes in the length of the ileum to be measured on the chart. The maximum contraction can be estimated to be 125 mm.

A 3 min cycle time was used to measure the effect of phentolamine on the contraction produced by the spasmogens, phentolamine being added 1 min before the spasmogens. The spasmogens were in contact with the ileum 1 min before washing out. The contraction heights were measured after 1 min contact time. A 4 min cycle time was used for the effect of phentolamine on the bradykinin relaxation.

RESULTS

Phentolamine reduction of the bradykinin relaxation after various spasmogens

Phentolamine, though not in the role of an α-adrenergic blocker, diminishes the bradykinin relaxation of the acetylcholine contracted guinea-pig ileum (chapter 3) and can also be shown to reduce the acetylcholine contraction. Since the type of contraction is involved in the regulation of the bradykinin relaxation (chapter 4) it was decided to examine the effect of phentolamine in the presence of a variety of spasmogens, in an attempt to discern if there were interactions between the spasmogens and phentolamine.

The bradykinin relaxations were measured in the presence of contraction of the ileum by convenient doses of acetylcholine, histamine, eledoisin and angiotensin, and in the presence of phentolamine added 1 min before the contraction by the spasmogens. A representative experiment using acetylcholine and phentolamine is shown in fig. 1. In the presence of contraction by acetylcholine, 20 ng/ml, it is observed that bradykinin at a concentration of 2 ng/ml produces a relaxation which is immediately followed by a small contraction above the elevated base-line. In the presence of phentolamine, 1 µg/ml, the acetylcholine contraction and also the bradykinin relaxation are reduced. At this phentolamine concentration it was noticed that the contraction which followed the bradykinin relaxation was never reduced,

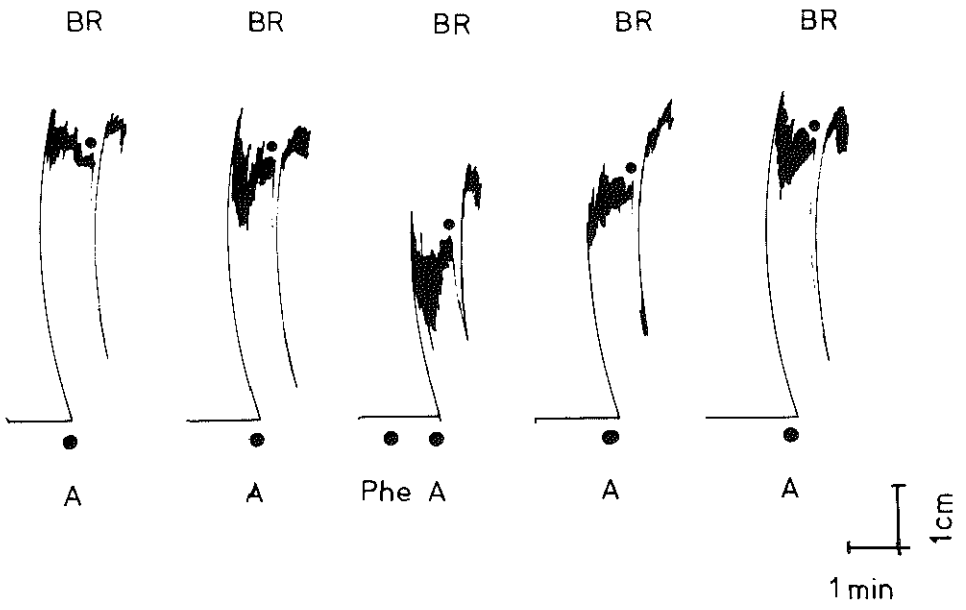

Fig. 1. The effect of phentolamine (Phe, 1 µg/ml) on the bradykinin (BR, 2 ng/ml) relaxation of the acetylcholine (A, 20 ng/ml) contracted guinea-pig ileum. Phentolamine was added 1 min before acetylcholine, and bradykinin 1 min after acetylcholine. Acetylcholine was in contact with the ileum 1 min 40 sec before washing out.

and on many occasions potentiated, as can be seen in the traces for fig. 1. The acetylcholine contraction, and the bradykinin relaxation and contraction recovered fully after washing with Tyrode solution. With acetylcholine, histamine and angiotensin, phentolamine (1 µg/ml) was used, however in the case of eledoisin 2 µg/ml was used. (The use of 2 µg/ml phentolamine, which was not detected until writing, was fortuitous, arising out of an error in dilution. The intended dilution was 1 µg/ml.) The results of the above experiments are shown in table 1.

Table 1

The influence of phentolamine on the bradykinin relaxation of the guinea-pig ileum in the presence of contraction by various spasmogens.

Spasmogens	BRADYKININ RELAXATION ± S.E.M. (mm)○ Control	Phentolamine △ treated	EXPECTED RELAXATIONS (mm)* Control	Phentolamine treated	% reduction of relaxation
Acetylcholine 20 ng/ml	39.0 ± 0.8	17.2 ± 0.5 (5)**	21.6	9.5	55.9
Histamine 40 ng/ml	35.1 ± 1.2	23.4 ± 0.7 (8)	41.9	27.9	33.3
Eledoisin 20 ng/ml	51.9 ± 0.9	35.3 ± 0.6 (8)	39.6	26.9	32.0
Angiotensin 20 ng/ml	21.4 ± 1.6	11.6 ± 1.0 (8)	14.3	7.8	45.5

○ Bradykinin was 2 ng/ml.

△ Phentolamine, added 1 min before the spasmogen, was 1 µg/ml, except with eledoisin 2 µg/ml.

* The 'EXPECTED RELAXATIONS' are the observed relaxations corrected to lie on the regression equations as found in table 1, chapter 4.

** Number of experiments in parentheses.

The bradykinin relaxations in the presence of the various spasmogens were then corrected so as to produce relaxations that lie on their respective common linear dose-response relationships, and taking the control responses found in table 1, chapter 4, at the specified spasmogen concentrations. These relaxations are termed the expected relaxations. The percentage of reduction of the bradykinin relaxation was then calculated. It can be seen that the greatest reduction by phentolamine is found in the presence of acetylcholine, followed by angiotensin, and histamine and eledoisin.

Phentolamine effects on the contraction of the ileum by various spasmogens

In table 2 can be seen the effect of phentolamine, 1 µg/ml, except with eledoisin, 2 µg/ml, on the acetylcholine, 20 ng/ml, histamine, 40 ng/ml, eledoisin, 20 ng/ml and angiotensin, 20 ng/ml contractions of the guinea-pig ileum. With the exception of eledoisin, contraction of the ileum by the same concentrations previously used was found to be reduced by phentolamine. The histamine contraction was reduced

Table 2

The influence of phentolamine on the acetylcholine, histamine, eledoisin and angiotensin contractions; and calculation of the theoretical bradykinin relaxation.

Spasmogens	CONTRACTION HEIGHTS (mm) Control ± S.E.M.	Phentolamine● ± S.E.M.	% reduction of contraction ± S.E.M.	% maximum contraction after phentolamine reduction.△	BRADYKININ RELAXATION○ Theoretical▲ (mm)	Expected* (mm)	% reduction of contraction (theoretical)
Acetylcholine 20 ng/ml	44.3 ± 0.9	23.9 ± 0.5	46.0 ± 2.1 (10)**	34.0	39.0	9.5	75.6
Histamine 40 ng/ml	46.1 ± 1.6	14.0 ± 1.0	69.8 ± 1.4 (8)	18.4	46.4	27.9	39.9
Eledoisin 20 ng/ml	55.3 ± 0.9	53.9 ± 1.6	NS (6)	85.1	39.6	26.9	32.0
Angiotensin 20 ng/ml	35.8 ± 3.3	19.8 ± 2.1	44.7 ± 1.0 (6)	52.3	14.5	7.8	46.2

○ Bradykinin was 2 ng/ml.

● Phentolamine 1 µg/ml, except with eledoisin 2 µg/ml.

△ The percentages of maximum contraction were for 20 ng/ml acetylcholine 63.0%, 40 ng/ml histamine 60.9%, 20 ng/ml eledoisin 85.1%, 20 ng/ml angiotensin 94.5% (see text for further details).

▲ The 'Theoretical bradykinin relaxations' are based on substitution of '% maximum contraction after phentolamine reduction' results in the respective regression equations (see table 1, chapter 4)

* The 'Expected relaxations' were taken from table 1.

** Numbers of experiments in parentheses.

greatest, followed by angiotensin, then acetylcholine. The percentage of maximum contraction of each of the spasmogens in the presence of phentolamine was then calculated using the information that 20 ng/ml acetylcholine produces a 63.0% maximum contraction, 40 ng/ml histamine 60.9%, 20 ng/ml eledoisin 85.1% and 20 ng/ml angiotensin 94.5%. These percentages, at the latter concentrations, comprised an integral part of the results needed to derive the regression equations (table 1, chapter 4). The theoretical bradykinin relaxations that should arise from these reduced contractions are then interpolated by substitution in the appropriate regression equation.

On comparison of the expected reduction of the bradykinin relaxation by phentolamine (table 1) with the theoretical reduction (table 2) is seen an increase from 55.9 to 75.6% with acetylcholine. With histamine there is a small increase, but this is probably not significant, while with eledoisin and angiotensin there is no diference between the expected and theoretical reductions of the bradykinin relaxation.

DISCUSSION

With the knowledge that phentolamine reduces the bradykinin relaxation of the acetylcholine contracted guinea-pig ileum (chapter 3), it was decided to examine whether reduction of the bradykinin relaxation was dependent on the spasmogen by which the ileum was contracted. The reduction of the bradykinin relaxation with phentolamine was thus measured after the ileum had been contracted by a variety of spasmogens. To obtain uniformity and afford a comparison of the various results after phentolamine treatment the bradykinin relaxations were corrected so as to lie on their respective linear dose-response relationships (chapter 4). These results were termed the expected relaxations. With histamine, eledoisin and angiotensin it can be seen that phentolamine reduces the bradykinin relaxation, though the magnitude of the expected reduction with each are not as great as that with acetylcholine. It appears that the bradykinin relaxation after eledoisin and histamine, which is modified to a smaller extent by changes in the spasmogen concentrations, is less reduced by phentolamine than that after acetylcholine. In the case of angiotensin where increasing concentrations up to a maximum contraction of the ileum have no effect on the bradykinin relaxation (chapter 4), there is an approximately 46% reduction of the bradykinin relaxation with phentolamine. It can be concluded that contraction of the ileum alone is unlikely to be a determining factor for the degree of phentolamine reduction of the bradykinin relaxation. There appears to be some specificity between the spasmogens themselves and the phentolamine reduction of the bradykinin relaxation.

The influence of phentolamine on the contraction of the ileum by the above spasmogens was also measured. This was conducted to find out if there were any particular relationships between the reduction in the spasmogen contraction and the bradykinin relaxation. It appeared that the largest reduction was with histamine (about 70%), while with angiotensin and acetylcholine the reduction was about 45%. The eledoisin contraction was not significantly reduced at the phentolamine concentration examined (twice that used with the other spasmogens). The latter results signify that phentolamine has both an anticholinergic and antihistaminic action, rather than an aspecific spasmolytic action on the isolated guinea-pig ileum. Since there exists linear

inverse relationships between the percentage of maximum contraction and the bradykinin relaxation, and, reduction of the spasmogen contraction by phentolamine influences the bradykinin relaxation, a theoretical reduction in the bradykinin relaxation after the different spasmogens was calculated. The theoretical reduction of the bradykinin relaxation with phentolamine in the presence of acetylcholine is larger than that in the presence of angiotensin, histamine or eledoisin respectively. The increased theoretical reduction of the bradykinin relaxation as compared with that of the expected reduction suggests that acetylcholine is in some way preventing phentolamine from exerting its full effect on the bradykinin relaxation. In the case of histamine, eledoisin and angiotensin there seems to be no particular wide deviations between the theoretical and expected relaxations.

Chapter 6

EFFECT OF SOME ANTI-INFLAMMATORY DRUGS ON THE BRADYKININ RELAXATION OF THE GUINEA-PIG ILEUM

SUMMARY

Indomethacin, sodium salicylate, phenylbutazone and amidopyrine have been examined for their effects on the bradykinin relaxation of the acetylcholine contracted guinea-pig ileum. At concentrations of the above drugs that are known to inhibit prostaglandin synthesis and also the bradykinin-induced bronchoconstriction of the guinea-pig lungs, there was generally no alteration of the bradykinin relaxation. In two isolated experiments the lag phase before the bradykinin contraction was markedly lengthened in the presence of indomethacin. At high concentrations of phenylbutazone the bradykinin relaxation and acetylcholine contraction were slightly depressed, while with high concentrations of amidopyrine the bradykinin relaxation was potentiated and acetylcholine contraction depressed. It is concluded for the acetylcholine contracted guinea-pig ileum that it is unlikely that bradykinin exerts its relaxing properties via a synthesis of prostaglandins or a mobilization of prostaglandin precursors, though it cannot be ruled out that prostaglandins are released, but do not cause movement of smooth muscle.

INTRODUCTION

Bradykinin has been implicated in forms of acute athritis, asthma, endotoxin shock and other inflammatory disturbances (Kellermeyer and Graham, 1968). The different anti-inflammatory drugs, initially found by empirical studies, have been examined extensively on the production, enzymatic destruction as well as on the direct effects of bradykinin. An extensive review of the subject has been written by Erdös (1966), Collier (1969) and Rocha e Silva (1970). In many cases smooth muscle preparations were used to study the potential anti-inflammatory drugs and gather information on the role of bradykinin in the inflammatory process. These preparations have unfortunately revealed many puzzling

effects of bradykinin and of drugs acting against bradykinin (Collier, 1969), such that a common denominator for the mode of action of bradykinin has not been found.

Some anti-inflammatory drugs have been shown, for example, to antagonize the bradykinin-induced bronchoconstriction of the guinea-pig lungs, both *in vivo* and *in vitro* (Graeff and Moog, 1964; Collier and Shortley, 1963), though the drugs were found to be inactive on the contraction of the guinea-pig ileum by bradykinin (Collier and Shortley, 1960; Jacques, 1965a). These same anti-inflammatory drugs have been shown to interfere with the action of slow reacting substance in anaphylaxis (SRS-A)(Berry and Collier, 1964), arachidonic acid (Berry, 1966) and slow reacting substance C (SRS-C)(Vargaftig et al., 1969), in guinea-pigs, rabbits and dogs. It has been demonstrated that bradykinin and SRS-A can release a substance, known as rabbit aorta contracting substance (RCS), from the isolated perfused sensitized guinea-pig lungs when challenged with antigen (Piper and Vane, 1969). Other substances released were histamine, SRS-A, PGE_2 and $PGF_{2\alpha}$. Piper and Vane also observed that release of RCS by bradykinin and SRS-A could be antagonized by aspirin-like drugs. With the finding that arachidonic acid, a precursor of prostaglandins, also releases RCS from the guinea-pig lungs, and that this release is antagonized by aspirin-like drugs (Vargaftig and Dao, 1971), there came the suggestion that RCS may be an intermediate in the synthesis of prostaglandins from arachidonic acid. It is now known that prostaglandin formation by homogenates of guinea-pig lung are antagonized by aspirin-like drugs (Vane, 1971); also, adrenaline-induced synthesis of prostaglandins in the isolated dog spleen (Ferreira et al., 1971); and, the production of prostaglandins induced by thrombin in human platelets (Smith and Willis, 1971).

Although it is unlikely that the bradykinin contraction of the guinea-pig ileum is mediated by the production of prostaglandins, it cannot be ruled out that bradykinin does not stimulate prostaglandin synthesis which has an, as yet, undetermined function. There is, however, no information available on the effects of anti-inflammatory drugs on the relaxation by bradykinin. It is possible that in organs where bradykinin elicits relaxation responses, the mechanism is indirect via a stimulation of prostaglandin synthesis. If this is the case then one might expect the responses to be antagonized by some of the

anti-inflammatory drugs. The present experiments were designed to examine the effects of some anti-inflammatory drugs on the bradykinin relaxation of the acetylcholine contracted guinea-pig ileum, in an attempt to find antagonistic activity.

MATERIALS AND METHODS

Lengths of guinea-pig ileum, 2 cm, were prepared according to the previously described method (chapter 2). A 4 min cycle time was used for the addition of acetylcholine and bradykinin, the acetylcholine being in contact with the ileum 1 min 40 sec and the bradykinin 40 sec before washing out. The anti-inflammatory drugs were added 1 min before the addition of acetylcholine. A 3 min cycle time was used to measure the effect of the anti-inflammatory drugs on the acetylcholine contraction. The anti-inflammatory drugs were added 1 min before the addition of acetylcholine, and the contraction height measured after the acetylcholine had been in contact with the ileum 1 min.

Drugs used additional to those in chapter 2: indomethacin (Merck, Sharp and Dohme, Holland), solubilized according to Northover (1971), phenylbutazone (CIBA-Geigy, Switzerland), amidopyrine (Aldrich-Europe, Belgium), sodium salicylate (Merck, Germany). All drugs, except indomethacin, were dissolved in Tyrode solution and doses correspond to their salts.

RESULTS

Anti-inflammatory drugs on the bradykinin relaxation

In table 1 can be seen a summary of the results that have been found with indomethacin, sodium salicylate, phenylbutazone and amidopyrine on both the bradykinin relaxation and acetylcholine contraction of the guinea-pig ileum. Indomethacin was present between 0.4 - 40 μg/ml. Concentrations between 0.1 - 1.0 μg/ml fall within the range giving inhibition of prostaglandin synthesis in homogenates of guinea-pig lung (Vane, 1971), and 0.37 - 4.0 μg/ml reduced the release of prostaglandins from the dog spleen (Ferreira et al., 1971). The sodium salicylate concentration (1000 μg/ml) used is higher than that required to inhibit

Table 1

The effect of some anti-inflammatory agents on the bradykinin relaxation and acetylcholine contraction of the guinea-pig ileum.

Drug	Dose[Δ] µg/ml	BRADYKININ RELAXATION[O] mm ± S.E.M.		%Blockade of acetylcholine contraction[Δ]
		Control	Treated	
Indomethacin	0.4	30.0 ± 0.8	29.0 ± 2.0 (4)**	NS
	4.0	33.6 ± 1.6	33.8 ± 2.3 (4)	NS
	8.0	44.3 ± 2.9	40.5 ± 2.3 (4)	NS
	40.0	36.8 ± 4.5	37.6 ± 4.4 (7)	NS
Sodium salicylate	1000.0	13.6 ± 0.7	12.8 ± 1.3 (6)	NS
Phenylbutazone	4.0	19.8 ± 0.6	18.8 ± 0.8 (4)	NS
	10.0	18.0 ± 1.2	19.3 ± 1.1 (4)	NS
	40.0	17.2 ± 0.3	15.9 ± 0.5 (7)*	7.5
Amidopyrine	40.0	24.8 ± 0.3	24.5 ± 0.5 (4)	NS
	100.0	23.8 ± 0.5	25.4 ± 0.4 (4)*	19.8

O Bradykinin, 2 ng/ml, was in contact with the ileum 40 sec. before washing out.

Δ The anti-inflammatory drugs were added 1 min before the acetylcholine, 20 ng/ml.

* Significant difference between the controls and treated experiments, $p \leq 0.05$ (Student's t-test). NS - Not significant.

** Numbers of experiments in parentheses.

prostaglandin synthesis in homogenates of guinea-pig lungs (Vane, 1971). Phenylbutazone was active at a concentration of 0.2 µg/ml in reducing the bradykinin-induced bronchoconstriction of the isolated guinea-pig lungs (Greaff and Moog, 1964). In the present experiments 4.0 - 40 µg/ml phenylbutazone was used. Amidopyrine has been found to be effective against the bradykinin-induced bronchoconstriction *in vivo*, though is 8 times less potent than indomethacin (Collier and Shortley, 1960, 1963). In the present experiments concentrations between 40.0 - 100.0 µg/ml were used.

It is seen that indomethacin does not reduce the bradykinin relaxation nor is there any significant effect on the acetylcholine contraction. In two isolated experiments, which could not be repeated, the time taken for the bradykinin contraction to appear after the bradykinin relaxation was greatly extended. After washing the ileum the

effect could still be observed. Further additions of indomethacin increased the lag time. There was no apparent effect on the magnitude of the bradykinin relaxation nor on the acetylcholine contraction. With sodium salicylate, 1000 µg/ml, no effect was found on either the bradykinin relaxation or the acetylcholine contraction. At low concentrations of phenylbutazone (4 - 10 µg/ml) there was no effect on the bradykinin relaxation or the acetylcholine contraction. At 40 µg/ml the bradykinin relaxation and the acetylcholine contraction were slightly reduced. With amidopyrine there was no effect at 40 µg/ml, but at 100 µg/ml was observed a slight potentiation of the bradykinin relaxation and a reduction of the acetylcholine contraction.

DISCUSSION

Some current ideas were presented in the introduction of this chapter as to the probable mode of action of a few anti-inflammatory agents. In particular, bradykinin was considered to release prostaglandin precursors in the perfused sensitized guinea-pig lung preparation (Piper and Vane, 1969), and this may be extendible to other smooth muscle preparations. Further it was considered that indomethacin and salicylate derivatives had the ability to inhibit prostaglandin synthesis in a variety of preparations (Vane, 1971; Ferreira et al., 1971; Smith and Willis, 1971). This inhibition has been suggested as a mechanism for their therapeutic actions (Vane, 1971; Collier, 1971).

The concentrations of anti-inflammatory drugs used in the present experiments are within the ranges used by the former authors, and, in some cases higher to secure inhibition of prostaglandin synthesis. Under no circumstances was there a reduction of the bradykinin relaxation with indomethacin, nor an effect on the acetylcholine contraction. It is curious that in two isolated experiments it was found that the length of time taken for the relaxation to recover was prolonged in the presence of indomethacin. We were, unfortunately, unable to repeat these experiments even after numerous attempts. Nevertheless, a reduction of the magnitude of the bradykinin relaxation was not found. These results indicated the unlikelyhood that the bradykinin relaxation of the acetylcholine contracted guinea-pig ileum is mediated by a *de novo* synthesis of prostaglandins, though it cannot be ruled out that

bradykinin does promote prostaglandin synthesis, but the effect is not measureable under these experimental conditions. In the case of phenylbutazone it is known that there exists a strong antagonism of SRS-A and the bradykinin-induced bronchoconstriction of the guinea-pig lung preparation, as is found with aspirin and indomethacin (Collier and Shortley, 1963; Berry and Collier, 1964). Of importance also, is that phenylbutazone can antagonize arachidonic acid contractions of the isolated guinea-pig ileum (Jacques, 1965a). These results point to a mechanism involving antagonism of prostaglandin synthesis or of arachidonic acid. No inhibition was found of the bradykinin relaxation at a low concentration, but there was a slight reduction at high concentrations. This, however, may be due to a direct depressant action of phenylbutazone, for the acetylcholine contraction was also reduced. It has been previously demonstrated that there is a linear inverse relationship between the acetylcholine contraction and the bradykinin relaxation, and it is possible to predict the bradykinin relaxation after contractions by acetylcholine (chapters 2,3,4). In this case the acetylcholine contraction is slightly reduced by phenylbutazone, hence theoretically there should be a greater reduction of the bradykinin relaxation after compensation for the effect on the acetylcholine contraction. Nevertheless, since there is such a small degree of blockade, and moreover indomethacin and sodium salicylate are inactive it is difficult to advance a mechanism involving prostaglandin synthesis for the bradykinin relaxation.

In the case of amidopyrine it is known that its anti-inflammatory activity can be antagonized by α-methyl DOPA and α-methyl-p-tyrosine (Jacques, 1965b) as well as by propranolol (Riesterer and Jacques, 1969). These authors consider that catecholamines contribute to the anti-inflammatory activity of amidopyrine, and that this mechanism may be active in smooth muscle. It is known that the bradykinin and SRS-A induced bronchoconstriction of the guinea-pig lungs is antagonized by amidopyrine, though the potency is less than that with aspirin (Collier and Shortley, 1960, 1963; Berry and Collier, 1964). At the same time amidopyrine is known to be an antagonist of arachidonic acid induced contractions of the isolated guinea-pig ileum (Jacques, 1965a). These results further implicate antagonism of prostaglandin synthesis or arachidonic acid as the basis of its therapeutic action. In the exper-

iments presented an effect with amidopyrine was only found at high concentrations, and suprisingly this was a potentiation of the relaxation. Also observed was a reduction of the acetylcholine contraction. It would seem likely, on the basis of what has been previously discussed concerning the acetylcholine contraction, that a decrease can account for the potentiation of the bradykinin relaxation. It is possible that released catecholamines reduce the acetylcholine contraction, though a more plausible explanation for amidopyrine would be via a direct mechanism on the smooth muscle. The prostaglandin release or precursor antagonism mechanisms are once again excluded.

REFERENCES

Berry, P.A., 1966, Slow reacting substance in anaphylaxis (SRS-A), its release, actions and antagonism, Ph.D. Thesis, Council of National Academic Awards, London.

Berry, P.A. and H.O.J. Collier, 1964, Bronchoconstrictor action and antagonism of a slow-contracting substance from anaphylaxis of the guinea-pig isolated lung, Brit. J. Pharmacol. Chemother. *23*, 201.

Collier, H.O.J., 1969, A pharmacological analysis of aspirin, Adv. Pharmacol. *7*, 333.

Collier, H.O.J., 1971, Prostaglandins and aspirin, Nature (London) *232*, 17.

Collier, H.O.J. and P.G. Shortley, 1960, Analgesic antipyretic drugs as antagonists of bradykinin, Brit. J. Pharmacol. Chemother. *15*, 601.

Collier, H.O.J. and P.G. Shortley, 1963, Antagonism by mefenamic and flufenamic acids of the bronchoconstrictor actions of kinins in the guinea-pig, Brit. J. Pharmacol. Chemother. *20*, 345.

Erdös, E.G., 1966, Hypotensive peptides: Bradykinin, kallidin and eledoisin, Adv. Pharmacol. *4*, 1.

Ferreira, S.H., S. Moncada and J.R. Vane, 1971, Indomethacin and aspirin abolish prostaglandin release from the spleen, Nature New Biology *231*, 237.

Graeff, K. and E. Moog, 1964, Vergleichende Untersuchungen über die bronchoconstrictorische und gefässconstrictorische Wirkung des Bradykinins, Histamins und Serotonins an isolierten Lungenpräparaten, Naunyn-Schmiedebergs Arch. exp. Path. u. Pharmakol. *248*, 204.

Jacques, R., 1965a, Suppression, by morphine and other analgesic compounds, of the smooth muscle contraction produced by arachidonic acid peroxide, Helv. Physiol. Acta *23*, 156.

Jacques, R., 1965b, Suppression, by α-methyl-DOPA and α-methyl-p-tyrosine of the anti-inflammatory action of aminopyrine, Helv. Physiol. Acta *23*, 311.

Kellermeyer, R.W. and R.C. Graham, Jr., 1968, Kinins - Possible physiologic and pathologic roles in man, New Eng. J. Med. *279*, 29.

Northover, B.J., 1971, Mechanism of the inhibitory action of indomethacin on smooth muscle, Brit. J. Pharmacol. Chemother. *41*, 540.

Piper, P.J. and J.R. Vane, 1969, Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs, Nature (London) *223*, 29.

Riesterer, L. and R. Jacques, 1969, Interference, by β-adrenergic blocking agents, with the anti-inflammatory action of various drugs, Helv. Physiol. Acta *26*, 287.

Rocha e Silva, M., 1970, In: Kinin Hormones, Springfield, Ill: C.C. Thomas Publishers.

Smith, J.B. and A.L. Willis, 1971, Aspirin selectively inhibits prostaglandin production in human platelets, Nature New Biology *231*, 235.

Vane, J.R., 1971, Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs, Nature New Biology *231*, 17.

Vargaftig, B.B. and N. Dao, 1971, Release of vasoactive susbtances from guinea-pig lungs by slow reacting substance C and arachidonic acid, Pharmacology *6*, 99.

Vargaftig, B.B., E.P. de Miranda and B. Lacoume, 1969, Inhibition by non steroidal anti-inflammatory agents of *in vivo* effects of slow reacting substance C, Nature (London) *222*, 883.

Chapter 7

POTENTIATION OF THE BIPHASIC BRADYKININ RESPONSE OF THE GUINEA-PIG ILEUM

SUMMARY

Cysteine, 2,3-dimercaptopropanol (BAL), α-thiol glycerol, and a synthetic pentapeptide (BPP_{5a}), identical with one of the bradykinin potentiating peptides from the venom of *Bothrops jararaca*, were examined on the bradykinin relaxation and contraction of the acetylcholine contracted guinea-pig ileum. Each of the substances was found to potentiate the bradykinin relaxation and contraction. Cysteine and BAL were up to ten times more potent at potentiating the bradykinin relaxation than the contraction. There was no potency difference for relaxation and contraction with α-thiol glycerol or BPP_{5a}. High concentrations of cysteine and BAL showed inconsistent effects, at some concentrations potentiating and others reducing the bradykinin responses. Cysteine at high concentrations potentiated, while BAL reduced the acetylcholine contraction. The possible existence of different receptors for bradykinin relaxation and contraction of the acetylcholine contracted guinea-pig ileum is discussed

INTRODUCTION

It is well documented that bradykinin can contract such organs as the guinea-pig ileum, the rabbit intestine, the rat uterus, stomach and intestines; while having a relaxant effect on the rat duodenum and colon (Rocha e Silva, 1970). The contractile effects of bradykinin on the guinea-pig ileum and the rat uterus can be shown to be potentiated by a variety of substances (Lewis, 1960; Picarelli et al., 1962). These include certain thiol compounds, such as cysteine (Lewis, 1960), 2,3-dimercaptopropanol (BAL) and α-thiol glycerol (α-TG)(Erdös and Wohler, 1963; Werle et al., 1964; Ferreira and Rocha e Silva, 1962), and also a potentiating factor (BPF) isolated from the venom of *Bothrops jararaca* (Ferreira, 1965). This was later shown to consist of nine different peptides (Ferreira et al., 1970). The pentapeptide (BPP_{5a}) has been subsequently synthesized and its potentiating activity confirmed.

It has been proposed that the mechanism for the potentiation by thiol compounds is inhibition of the enzymes that destroy bradykinin, i.e. kininases (Lewis, 1960; Picarelli et al., 1962; Ferreira and Rocha e Silva, 1962; Sherman and Gautieri, 1969). There has, however, been an accumulation of evidence arguing against enzyme inhibition as the most important *in vitro* mechanism. Direct potentiating mechanisms have been proposed (Cirstea, 1965; Doleschel and Auerswald, 1966; Auerswald and Doleschel, 1967; Tewksbury, 1967). More recently a neurogenic mechanism has been suggested for the potentiation of the bradykinin contraction of the guinea-pig ileum by cysteine (Potter and Walaszek, 1972). They propose that potentiation proceeds via a facilitation of acetylcholine release from the nerve ending. There seems to be little work pertaining to the relaxation effects of bradykinin on intestinal smooth muscle, though one communication has examined the potentiation on the rat duodenum and terminal ileum by BAL, BPF and BPP_{5a}. (Camargo and Ferreira, 1971). The finding that bradykinin can relax the acetylcholine contracted guinea-pig ileum (chapter 2) has prompted the present investigation into the possibility of potentiating this bradykinin response.

MATERIALS AND METHODS

The isolated guinea-pig ileum preparations were set-up according to a previously described method (chapter 2). A 4 min cycle time was used for the addition of acetylcholine and bradykinin, acetylcholine being in contact with the ileum 1 min 40 sec and bradykinin 40 sec before washing out. The potentiating agents were added 1 min before the addition of acetylcholine. A 3 min cycle time was used to study the effect of the potentiating agents on the acetylcholine contraction, the agents being added 1 min before the addition of acetylcholine. The acetylcholine was in contact with the ileum 1 min before washing out. The height of the contraction was measured at the termination of the 1 min contact time.

Drugs used in addition to those in chapter 2: cysteine, 2,3-dimercaptopropanol (BAL), α-thiol glycerol (Merck, Germany). The 10^{-2}M cysteine solution had a pH of 7.2, and thus the inhibitory effects of acid cysteine solutions on kininases, as found by Aarsen and Kemp (1962), were avoided. The synthetic pentapeptide, pyroglutamyl-lys-try-ala-pro

(BPP_{5a}), corresponds to one of the nine peptides responsible for the pharmacological actions of bradykinin potentiating factor (BPF) isolated from the venom of *Bothrops jararaca* (Ferreira et al., 1970). BPP_{5a} was synthetically prepared by Ir. E.W.B. de Leer and Mr. C. Olieman and I gratefully acknowledge a specimen from Prof. Dr. H.C. Beyerman, Department of Organic Chemistry, T.H. Delft. A sample of Sephadex G 50 fractionated *Bothrops jararaca* venom, containing the low molecular weight peptides (BPF), was kindly donated by Dr. N. Bhargava, Department of Pharmacology, Organon, Holland.

RESULTS

Cysteine and the bradykinin response

Representative traces for the effect of cysteine on the bradykinin responses are shown in fig. 1. It is observed that with cysteine,

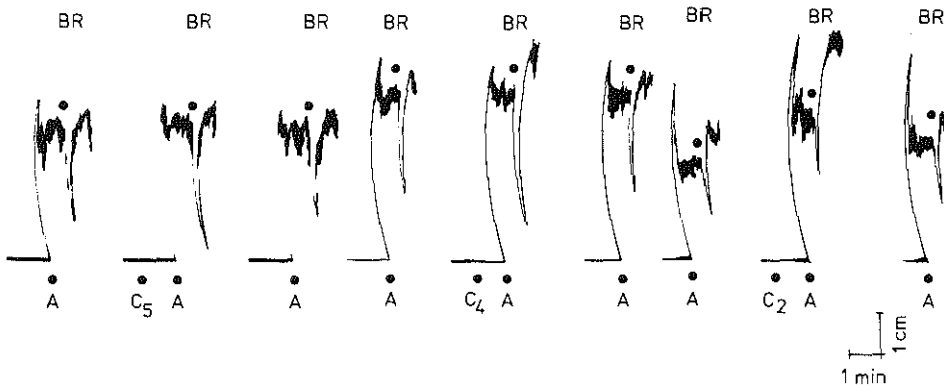

Fig. 1. The effect of cysteine ($C_{5,4,2}$, $10^{-5,-4,-2}$M respectively) on the bradykinin (BR, 2 ng/ml) response of the acetylcholine (A, 20 ng/ml) contracted guinea-pig ileum. Cysteine was added 1 min before acetylcholine, and bradykinin 1 min after acetylcholine. Acetylcholine was in contact with the ileum 1 min 40 sec before washing out. The recorder module sensitivities are the same for each of the horizontal groups, however the base-lines before the acetylcholine contraction are different, thus the acetylcholine contraction heights are not comparable in these traces. Base-line changes were produced by vertical displacement of the transducer module.

10^{-5}M, only the relaxation component is potentiated there being no potentiation of the bradykinin contraction. At 10^{-4}M cysteine both the relaxation and contraction responses were potentiated. With a high concentration of cysteine, 10^{-2}M, it can be seen that the bradykinin relaxation is slightly reduced, while the contraction is markedly potentiated. At this concentration the acetylcholine contraction can be seen to be slightly potentiated. With the traces shown the recorder module sensitivities were the same for each experiment, though the base-lines before acetylcholine contraction were different for each group. Base-line changes were produced by vertical displacement of the transducer module. It was necessary to use different base-lines since, at the sensitivity chosen the pen excursion would be too small for inclusion of the complete bradykinin response. The effect of some cysteine concentrations, 10^{-7}- 10^{-2}M, on the bradykinin response is shown in table 1. Cysteine potentiated the bradykinin contraction at concentrat-

Table 1

Effect of cysteine on the bradykinin responses of the acetylcholine contracted guinea-pig ileum.

Cysteine$^{\Delta}$ concentration (M)	BRADYKININ CONTRACTION$^{\circ}$ mm ± S.E.M.			BRADYKININ RELAXATION$^{\circ}$ mm ± S.E.M.		
	Control	Cysteine	% Controls	Control	Cysteine	% Controls
10^{-7}	-		-	18.3 ± 1.3	18.5 ± 1.4 (6)**	NS
10^{-6}	12.0 ± 0.7	11.8 ± 0.8 (10)	NS	26.7 ± 0.9	27.4 ± 0.5 (11)	NS
10^{-5}	11.0 ± 0.8	11.8 ± 0.9 (6)	NS	21.9 ± 1.3	25.8 ± 1.6 (12)*	118
10^{-4}	11.2 ± 0.8	19.0 ± 1.5 (6)*	170	25.9 ± 1.0	32.7 ± 1.2 (14)*	122
10^{-3}	13.4 ± 0.5	35.5 ± 2.1 (8)*	265	27.0 ± 2.1	28.6 ± 2.3 (12)	NS
10^{-2}	14.1 ± 1.0	27.6 ± 1.8 (8)*	196	24.5 ± 1.2	20.6 ± 1.1 (10)*	84

$^{\circ}$ Bradykinin was 2 ng/ml.

$^{\Delta}$ Cysteine was added 1 min before acetylcholine, 20 ng/ml.

* Significant difference between controls and cysteine experiments, $p \leq 0.05$ (Student's t-test), NS - Not significant.

** Numbers of experiments in parentheses.

ions between 10^{-4}- 10^{-2}M, and also the relaxation at 10^{-5}- 10^{-4}M. There appeared to be no significant difference between the controls and the cysteine treated ilea at 10^{-3}M for the bradykinin relaxation. At 10^{-2}M

cysteine the bradykinin relaxation was seen to be reduced. The bradykinin contraction seemed to show a greater maximum potentiation than the relaxation, though these quantities are not strictly comparable.

2,3-dimercaptopropanolol (BAL) and the bradykinin response

In table 2 can be seen the effect of BAL, 10^{-7}- 10^{-3}M, on the bradykinin response. BAL can be observed to potentiate the contractile effects of bradykinin at concentrations between 10^{-5}- 10^{-4}M, while at a concentration of 10^{-3}M the contraction was significantly reduced. Potentiation of the relaxation response was observed between 10^{-6}- 10^{-5}M. No other significant differences were seen between the controls and the BAL treated ilea. The bradykinin contraction appeared to show a greater maximum potentiation than the relaxation, though these quantities are not strictly comparable.

Table 2

Effect of BAL on the bradykinin responses of the acetylcholine contracted guinea-pig ileum.

BAL concentration (M)	BRADYKININ CONTRACTION° mm ± S.E.M.			BRADYKININ RELAXATION Δ mm ± S.E.M.		
	Control	BAL	% Control	Control	BAL	% Control
10^{-7}	3.2 ± 0.9	4.8 ± 1.1 (5)**	NS	22.4 ± 1.0	21.8 ± 0.3 (5)	NS
10^{-6}	4.1 ± 0.3	4.5 ± 0.4 (6)	NS	23.9 ± 1.3	28.9 ± 1.6 (6)*	121
10^{-5}	2.0 ± 0.7	8.7 ± 1.1 (11)*	435	20.5 ± 0.5	24.3 ± 0.4 (11)*	118
10^{-4}	5.2 ± 1.1	17.9 ± 1.3 (9)*	344	23.0 ± 0.8	23.9 ± 0.8 (9)	NS
10^{-3}	5.9 ± 1.3	2.3 ± 0.7 (7)*	39	17.3 ± 0.8	16.8 ± 0.9 (7)	NS

° Bradykinin was 2 ng/ml.

Δ BAL was added 1 min before acetylcholine, 20 ng/ml.

* Significant difference between controls and BAL experiments, $p \leq 0.05$ (Student's t-test), NS - Not significant.

** Numbers of experiments in parentheses.

α-thiol glycerol (α-TG) and the bradykinin response

The effect of a range of concentrations of α-TG is seen in table 3.

Table 3

Effect of α-thiol glycerol on the bradykinin responses of the acetylcholine contracted guinea-pig ileum.

α-thiol glycerol° concentration (M)	BRADYKININ CONTRACTION△ mm ± S.E.M.			BRADYKININ RELAXATION mm ± S.E.M.		
	Control	α-TG	% Control	Control	α-TG	% Control
10^{-4}	11.6 ± 1.0	11.9 ± 1.0 (8)**	NS	22.3 ± 2.9	21.4 ± 3.0 (8)	NS
10^{-3}	11.6 ± 1.2	13.0 ± 1.2 (9)	NS	20.9 ± 2.3	26.5 ± 3.0 (9)	NS
5×10^{-3}	10.8 ± 3.6	19.2 ± 1.8 (6)*	178	27.3 ± 1.8	39.1 ± 4.3 (7)*	144
10^{-2}	10.6 ± 1.2	17.7 ± 1.7 (11)*	167	17.1 ± 1.5	26.6 ± 2.7 (12)*	143

△ Bradykinin was 2 ng/ml.

° α-TG was added 1 min before acetylcholine, 20 ng/ml.

* Significant difference between controls and α-TG experiments, $p \leq 0.05$ (Student's t-test), NS - Not significant.

** Numbers of experiments in parentheses.

Both the contraction and relaxation responses were potentiated at concentrations above 5×10^{-3}M, and further both showed similar magnitudes of potentiation.

Synthetic bradykinin potentiating factor (BPP_{5a}) and the bradykinin response

In fig. 2 can be seen representative traces for the effect of BPP_{5a} on the bradykinin response. It is observed that BPP_{5a}, 2×10^{-6}M, causes both a potentiation of the bradykinin contraction and relaxation. At this concentration there is no effect on the acetylcholine contraction. Table 4 shows the effect of increasing concentrations of BPP_{5a} on the bradykinin responses. Both the contraction and relaxation responses are potentiated at concentrations above 10^{-7}M, with a maximum at 2×10^{-6}M. The bradykinin relaxation appeared to show a greater percentage maximum potentiation than the contraction, though these quantities are not strictly comparable. Both the bradykinin relaxation and contraction responses were potentiated by BPF at a threshold concentration of 2 µg/ml.

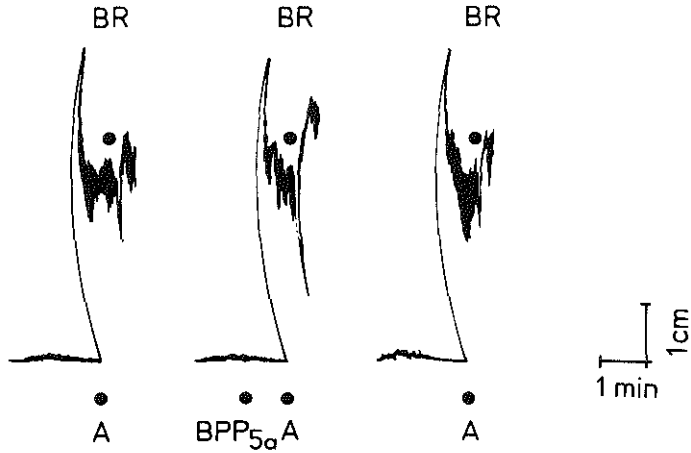

Fig. 2. The effect of BPP_{5a} (2×10^{-6} M) on the bradykinin (BR, 2 ng/ml) response of the acetylcholine (A, 20 ng/ml) contracted guinea-pig ileum. BPP_{5a} was added 1 min before acetylcholine, and bradykinin 1 min after acetylcholine. Acetylcholine was in contact with the ileum 1 min 40 sec before washing out.

Table 4

Effect of BPP_{5a} on the bradykinin responses of the acetylcholine contracted guinea-pig ileum.

BPP_{5a} ○ concentration (M)	BRADYKININ CONTRACTION △ mm ± S.E.M.			BRADYKININ RELAXATION mm ± S.E.M.		
	Control	BPP_{5a}	% Control	Control	BPP_{5a}	% Control
10^{-7}	10.8 ± 0.3	11.2 ± 0.5 (5)**	NS	12.0 ± 0.5	12.6 ± 0.6 (5)	NS
2×10^{-7}	11.5 ± 0.6	15.3 ± 0.9 (5)*	133	12.3 ± 0.6	16.3 ± 0.5 (5)*	133
4×10^{-7}	9.6 ± 0.7	14.6 ± 0.9 (5)*	152	11.2 ± 0.4	18.4 ± 0.8 (5)*	165
2×10^{-6}	7.8 ± 0.4	13.4 ± 0.8 (5)*	172	9.6 ± 0.4	19.8 ± 0.6 (5)*	206
4×10^{-6}	7.6 ± 0.7	12.7 ± 0.6 (5)*	167	10.8 ± 0.5	22.0 ± 0.3 (5)*	204

△ Bradykinin was 2 ng/ml.

○ BPP_{5a} was added 1 min before acetylcholine, 20 ng/ml.

* Significant difference between controls and BPP_{5a} experiments, $p \leq 0.01$ (Student's t-test), NS - Not significant.

** Numbers of experiments in parentheses.

Various potentiating agents and the acetylcholine contraction

Previously (chapter 2,4) it was shown that an increase in the concentration of challenging acetylcholine resulted in a decrease of the bradykinin relaxation. With this information it has been possible to predict the magnitude of the bradykinin relaxation after any contraction by acetylcholine (chapter 3,4). It would thus seem possible to predict the magnitude of the bradykinin relaxation after potentiation or reduction of the acetylcholine contraction. In table 5 can be seen the

Table 5

Effect of various thiol compounds on the acetylcholine contraction of the guinea-pig ileum.

Thiol compound concentration (M)	ACETYLCHOLINE CONTRACTION Δ mm ± S.E.M.			
	Control	Cysteine	Control	BAL
10^{-6}	-	-	46.6 ± 1.5	46.4 ± 1.7 (7)
10^{-5}	-	-	41.7 ± 1.2	41.3 ± 0.9 (9)
10^{-4}	44.1 ± 0.7	45.3 ± 0.2 (5)**	43.8 ± 1.6	42.7 ± 1.7 (8)
10^{-3}	43.8 ± 0.9	47.4 ± 1.3 (6)*	35.8 ± 2.2	27.9 ± 2.4 (7)*
10^{-2}	44.0 ± 0.9	49.9 ± 0.9 (5)*	-	-

Δ Acetylcholine was 20 ng/ml.

o The thiol compound was added 1 min before acetylcholine.

* Significant difference between controls and thiol compound experiments, $p \leq 0.05$ (Student's t-test).

** Numbers of experiments in parentheses.

effect of cysteine and BAL on the acetylcholine contraction. It is observed that cysteine at 10^{-3} and 10^{-2}M potentiates the acetylcholine contraction, but there appears to be no direct effect at lower concentrations. A significant reduction of the acetylcholine contraction is seen with BAL, though only at 10^{-3}M, there being no effect at lower concentrations. These actions of cysteine and BAL on the acetylcholine

contraction should give rise with cysteine to a decrease in the bradykinin relaxation, and with BAL an increase in the bradykinin relaxation. α-TG and BPP_{5a} did not affect the acetylcholine contraction at any of the concentrations examined.

DISCUSSION

It would appear that the component of relaxation by bradykinin of the acetylcholine contracted guinea-pig ileum is up to ten times more sensitive to potentiation by cysteine than the contraction. By the use of the various concentrations of cysteine it is possible to potentiate either the relaxation or contraction response without effect on the other. Previously (chapter 2,4,) it was seen that an increase in the concentration of acetylcholine resulted in a decrease in the relaxation by a fixed dose of bradykinin. These latter findings were quantified such that it was possible to predict the magnitude of the bradykinin relaxation after any contraction involving acetylcholine (chapter 3,4). A potentiation of the acetylcholine contraction by cysteine would be expected to result in a decreased relaxation by bradykinin, as is indeed observed. This indirect effect is likely to play a major role in reducing the direct potentiation by cysteine, though probably there is also a direct depressant action of cysteine at these high concentrations.

The bradykinin relaxation is up to ten times more sensitive to potentiation by BAL than the contraction, and the threshold for potentiation of the bradykinin relaxation is seen to be ten times smaller than that for cysteine. It appears possible to use BAL selectively to potentiate either the contraction or relaxation responses without effect on the other. In a manner similar to that with cysteine the effect of BAL on the acetylcholine contraction must be taken into consideration. At 10^{-3}M BAL a reduction of the contraction is observed. This would be expected to lead to a potentiation of the bradykinin relaxation, but is not found. It is likely that at these high BAL concentrations there is a direct depressant action masking the potentiation effects.

The finding that BAL at 10^{-6}M potentiates only the bradykinin relaxation without effect on the bradykinin contraction is at variance with the results reported for the rat and cat intestines (Camargo and Ferreira, 1971; Alabaster and Bakhle, 1972). In table 6 is depicted a summary of

Table 6

Differences in the effects of BAL and BPP_{5a} on the bradykinin response of various isolated intestinal preparations.

isolated organ	BAL bradykinin responses contraction	BAL bradykinin responses relaxation	BPP_{5a} bradykinin responses contraction	BPP_{5a} bradykinin responses relaxation
acetylcholine contracted guinea-pig ileum	0	+	+	+
rat duodenum *	-	0	+	0
rat terminal ileum *	-	0+	+	0
cat terminal ileum **	+	no results available	0	no results available

+ potentiation, 0 no effect, - blockade

*data from Camargo & Ferreira (1971).

** data from Alabaster & Bakhle (1972).

the findings. Camargo and Ferreira demonstrated that BAL (approximately 10^{-6}M) had no effect on the bradykinin relaxation of the rat duodenum, but caused a slight potentiation on the rat terminal ileum. Further they found that BAL prevented the bradykinin contraction of these preparations. On the other hand it has recently been reported (Alabaster and Bakhle, 1972) that the bradykinin contraction of the cat jejunum and terminal ileum was potentiated by BAL (approximately 10^{-4}M) in some of their experiments. It would appear that there exists species and organ differences with regard to the action of BAL on the bradykinin responses of intestinal smooth muscle preparations.

The results with BPP_{5a} mirror those found with BPF on the acetylcholine contracted guinea-pig ileum, and the rat duodenum (Camargo and Ferreira, 1971). With BPP_{5a} both the bradykinin relaxation and contraction responses of the guinea-pig ileum were potentiated, with a similar threshold concentration (2×10^{-7}M). There was no effect on the acetylcholine contraction at the concentrations examined. These results are at variance, in some respects, with those of Camargo and Ferreira (1971), see table 6, who found that BPP_{5a} (approximately 10^{-5}M) had no

effect on the bradykinin relaxation of the rat duodenum and terminal ileum, though potentiated the bradykinin contraction. In contrast BPP_{5a} (approximately 10^{-6}M) was shown to have no effect on the bradykinin contraction of the cat jejunum (Alabaster and Bakhle, 1972). It seems that there are also species differences for the potentiation of bradykinin responses by BPF or BPP_{5a}.

For α-thiol glycerol there is a threshold potentiation for both relaxation and contraction at about 5×10^{-3}M. There appears to be neither discrimination between potentiation of relaxation or contraction nor effects on the acetylcholine contraction. In these respects the action of BPP_{5a} is similar. It is possible that α-TG and BPP_{5a} act as potentiators of the bradykinin response of the guinea-pig ileum because of their inhibition of kinin breakdown, though the possibility of blockade of a single receptor or a non-discriminative action on multiple receptors cannot be excluded.

The potentiation of the bradykinin contraction of the acetylcholine contracted guinea-pig ileum is unlikely to be due to a cysteine-facilitated release of acetylcholine from the nerve endings of the guinea-pig ileum, as suggested by Potter and Walaszek (1972), since increasing concentrations of acetylcholine had no influence on the bradykinin contraction, and even reduced the bradykinin relaxation (chapter 4). Although exogenously applied acetylcholine was found to be ineffective at potentiating the bradykinin contraction, this does not rule out the possibility of a neurogenic mechanism on the ileum with normal tone. Nevertheless, these findings indicate the differences in the mechanisms of the bradykinin relaxation and contraction of the guinea-pig ileum that exist. There have been observations hinting at the existence of separate receptors on the guinea-pig ileum for bradykinin (chapter 3,5). It was reported that the bradykinin relaxation of the acetylcholine contracted guinea-pig ileum was reduced by phentolamine, while no reduction in the bradykinin contraction was observed; moreover, frequently a potentiation resulted. If there were one receptor common to both relaxation and contaction responses, one might expect both responses to be reduced by phentolamine, which is not found. It cannot be completely certain that bradykinin receptors are involved, since phentolamine and also the potentiators may have their specificity on a post-receptor mechanism. Although the results suggesting duality of the bradykinin receptors for relaxation

and contraction on the rat intestines (Camargo and Ferreira, 1971) could not be repeated for the guinea-pig ileum, the present results do show discriminative effects for cysteine and BAL on the bradykinin response. In view of these results, it is conceivable that two discrete receptors for bradykinin exist on the guinea-pig ileum. These results do not rule out the possibility of a mechanism involving a single receptor with two efferent post-receptor pathways. With high concentrations of cysteine, however, the possibility cannot be eliminated that the direct effect on bradykinin was secondary, the primary effect being indirect on the acetylcholine contraction. With BAL this is unlikely since the observed effect on bradykinin is contrary to that expected on the basis of the influence on the acetylcholine contraction.

REFERENCES

Aarsen, P.N. and A. Kemp, 1962, Influence of metals on the activity of a bradykinin-destroying enzyme kininase, Brit. J. Pharmacol. Chemother. *19*, 442.

Alabaster, V.A. and Y.S. Bakhle, 1972, The inactivation of bradykinin in the pulmonary circulation of isolated lungs, Brit, J. Pharmacol. Chemother. *45*, 299.

Auerswald, W. and W. Doleschel, 1967, On the potentiation of kinins by sulphydryl compounds, Arch. Intern. Pharmacodyn. Ther. *168*, 188.

Camargo, A. and S.H. Ferreira, 1971, Action of bradykinin potentiating factor (BPF) and dimercaprol (BAL) on the response to bradykinin of isolated preparations of rat intestines, Brit. J. Pharmacol. Chemother. *42*, 305.

Cirstea, M., 1965, Potentiation of some bradykinin effects by thiol compounds, Brit. J. Pharmacol. Chemother. *25*, 405.

Doleschel, W. and W. Auerswald, 1966, On the mechanism of potentiation of kinins by inhibitors of the fibrinolytic system, Experientia (Basel) *22*, 540.

Erdös, E.G. and J.R. Wohler, 1963, Inhibition *in vivo* of the enzymatic inactivation of bradykinin and kallidin, Biochem. Pharmacol. *12*, 1193.

Ferreira, S.H., 1965, Bradykinin potentiating factor (BPF) present in the venom of *Bothrops jararaca*, Brit. J. Pharmacol. Chemother. *24*, 163.

Ferreira, S.H., D.C. Bartelt and L.J. Greene, 1970, Isolation of bradykinin potentiating peptides from *Bothrops jararaca* venom, Biochemistry *9*, 2583.

Ferreira, S.H. and M. Rocha e Silva, 1962, Potentiation of bradykinin by dimercaptopropanol (BAL) and other inhibitors of its destroying enzyme in plasma, Biochem. Pharmacol. *11*, 1123.

Lewis, G.P., 1960, Active polypeptides derived from plasma proteins, Physiol. Rev. *40*, 647.

Picarelli, Z.P., O.B. Henriques and M.C.F. Oliveira, 1962, Potentiation of bradykinin action on smooth muscle by cysteine, Experientia (Basel) *15*, 77.

Potter, D.E. and E.J. Walaszek, 1972, Potentiation of the bradykinin response by cysteine: Mechanism of action, Arch. Intern. Pharmacodyn. Ther. *197*, 338.

Rocha e Silva, M., 1970, In: Kinin Hormones, Springfield, Ill: C.C. Thomas Publishers.

Sherman, W.T. and R.F. Gautieri, 1969, Cardiovascular and gastrointestinal effects of bradykinin and its potentiation by thiols in rats, J. Pharm. Sci. *58*, 971.

Tewksbury, D.A., 1968, Studies on the mechanism of bradykinin potentiation, Arch. Intern. Pharmacodyn. Ther. *173*, 426.

Werle, E., K. Hochstrasser, I. Trautschold and G. Leysath, 1964, Wirkung von Thioglykolsäure auf Kininogen und Kininase, Hoppe-Seylers Z. physiol. Chem. *337*, 286.

SUMMARY

The investigations presented in this thesis are concerned with the conditions for and the characteristics of the bradykinin induced relaxation of the isolated guinea-pig ileum.

The isolated guinea-pig ileum with normal tone when challenged by bradykinin produces only contraction responses. If, however, the ileum is first challenged by acetylcholine giving approximately 60% maximum contraction, bradykinin caused a relaxation immediately followed by a contraction. The biphasic response to bradykinin was also found when acetylcholine was replaced by histamine, eledoisin or prostaglandin $F_{2\alpha}$. A biphasic response was also produced during transmural electrical stimulation. The conditions for the bradykinin relaxation were not found in the presence of contraction of the ileum by bradykinin itself, or potassium or barium chloride. Under the conditions where bradykinin produced a biphasic response, acetylcholine, histamine, eledoisin, prostaglandin $F_{2\alpha}$ and lysine-vasopressin only contracted the ileum, while adrenaline, noradrenaline, oxytocin, calcium and magnesium chloride only relaxed the ileum. On increasing the percentage of maximum contraction with acetylcholine, a linear inverse relationship with the bradykinin-induced relaxation was found. Repeated single doses of bradykinin were not found to produce a tachyphylactic relaxation response. The relaxing effect of bradykinin is more likely to be due to a direct effect on the muscle cell membrane than to a release of a mediator or to the blockade of a receptor mediating contraction.

The possibility existed that bradykinin releases stored catecholamines or that bradykinin can in some way stimulate the adrenergic receptors with the result of relaxation of the guinea-pig ileum. The effect of various α- and β-adrenergic blockers have been examined on both the bradykinin and adrenaline relaxation of the guinea-pig ileum, as a means to substantiate this hypothesis. The α-adrenergic blocker piperoxan potentiated, while phentolamine, also an α-adrenergic blocker, reduced the bradykinin relaxation. Both reduced the acetylcholine contraction, but had no effect on the bradykinin relaxation. The bradykinin relaxation of the guinea-pig ileum was up to ten times more sensitive to phentolamine than that of the rat duodenum. The β-adrenergic blocker propranolol potentiated the bradykinin relaxation and

reduced the acetylcholine contraction. Sotalol, also a β-adrenergic blocker, was in these respects less potent than propranolol. The adrenaline relaxation was partially blocked by propranolol, but almost completely by sotalol. A combination of phentolamine and propranolol slightly potentiated the bradykinin relaxation, but partially blocked the adrenaline relaxation. It can be concluded that the bradykinin relaxation is not due to a direct action on either the α- or β-adrenergic receptors of the guinea-pig ileum. Moreover the potentiation of the bradykinin relaxation by piperoxan and propranolol is probably indirect via a reduction of the acetylcholine contraction. The action of phentolamine in reducing the bradykinin relaxation cannot be explained by an effect on the acetylcholine contraction. For the isolated guinea-pig ileum only β-adrenergic stimulation mediates relaxation with adrenaline.

The bradykinin relaxation of the acetylcholine contracted guinea-pig ileum is decreased by increasing the concentration of acetylcholine. This finding prompted an investigation to determine whether other spasmogens can reduce the bradykinin relaxation, and the mechanisms involved. Linear inverse relationships were found between both the percentages of maximum contraction and concentrations of acetylcholine, histamine and eledoisin, and the bradykinin relaxation. Acetylcholine showed a steeper regression equation slope than eledoisin or histamine. No relationship existed for angiotensin, though low non-spasmogenic doses in the presence of acetylcholine reduced the bradykinin relaxation. Morphine and atropine reduced the angiotensin contraction, and potentiated the bradykinin relaxation in its presence. Only atropine reduced the acetylcholine contraction and potentiated the bradykinin relaxation in its presence. Neither morphine nor atropine had any effect on the bradykinin reduction of its own relaxation responses. The magnitude of the bradykinin relaxation of the guinea-pig ileum is dependent on the manner of stimulation, rather than on the state of contraction; and, is also sensitive to regulation by agents that have their actions on the parasympathetic system. Angiotensin reduces the bradykinin relaxation by a mechanism involving its indirect parasympathomimetic effects. In contrast, the bradykinin reduction of its own relaxation is not via a parasympathetic mechanism, but is probably a direct action.

It has also been considered that the magnitude of the reduction of the bradykinin relaxation by phentolamine may vary in the presence of different spasmogens. Phentolamine was shown to reduce the bradykinin relaxation most potently after acetylcholine, followed by angiotensin, then histamine and eledoisin. Phentolamine invariably reduced the bradykinin relaxation, but the amount of reduction was dependent on the initial spasmogen. In this respect phentolamine reduced the histamine contraction greatest, followed by acetylcholine and angiotensin. The eledoisin response was not modified by phentolamine.

The possibility that bradykinin releases prostaglandins (*de novo* synthesis) in the guinea-pig ileum, which may result in relaxation has been considered as a possible indirect mechanism for the bradykinin relaxation. Some anti-inflammatory agents known to inhibit either prostaglandin synthesis or block the bradykinin bronchoconstriction of the guinea-pig lungs have been examined for their effects on the bradykinin relaxation. At concentrations of indomethacin, sodium salicylate, phenylbutazone and amidopyrine known to inhibit prostaglandin synthesis there was no alteration in the bradykinin relaxation. In two independent experiments using indomethacin the lag period before the bradykinin contraction was markedly extended. At high concentrations of phenylbutazone the bradykinin relaxation and the acetylcholine contraction were slightly depressed, while high concentrations of amidopyrine potentiated the bradykinin relaxation and depressed the acetylcholine contraction. It is concluded that it is unlikely that bradykinin exerts its relaxing properties via a synthesis of prostaglandins or mobilization of prostaglandin precursors, though it cannot be ruled out that bradykinin does stimulate prostaglandin synthesis, but the resulting prostaglandins have no effect on the tone of the guinea-pig ileum.

Bradykinin responses have been shown to be potentiated by a number of substances. Accordingly the possibility of potentiation has been investigated on the bradykinin relaxation of the acetylcholine contracted guinea-pig ileum. The substances cysteine, 2,3-dimercaptopropanol (BAL), α-thiolglycerol, and a synthetic pentapeptide (BPP_{5a}), identical with one of the bradykinin potentiating peptides (BPF) isolated from the venom of *Bothrops jararaca*, have been examined on both the bradykinin relaxation and contraction. Each of the substances was found to

potentiate the bradykinin relaxation and contraction. Cysteine and BAL were up to ten times more potent at potentiating the bradykinin relaxation than the contraction. There was no potency difference for α-thiolglycerol or BPP_{5a} with relaxation and contraction. High concentrations of cysteine and BAL showed inconsistent effects, at some concentrations potentiating and others reducing the bradykinin responses. At high concentrations, cysteine potentiated, while BAL reduced the acetylcholine contraction. α-Thiolglycerol and BPP_{5a} had no effect at any concentration examined. The possible existence of different receptors for the bradykinin induced relaxation and contraction of the acetylcholine contracted guinea-pig ileum is discussed.

SAMENVATTING

In dit proefschrift zijn onderzoekingen beschreven waarvan het doel was, de door bradykinine opgewekte relaxatie van het geïsoleerde cavia ileum te karakteriseren en de voorwaarden waaronder deze optreedt te bepalen.

Toevoeging van bradykinine aan een geïsoleerd cavia ileum met normale tonus veroorzaakt een contractie. In aanwezigheid van acetylcholine in een concentratie die 60% van de maximale contractie van het ileum geeft, werd na toevoeging van bradykinine een relaxatie verkregen, direkt gevolgd door een contractie. Deze bifasische respons van bradykinine werd eveneens verkregen indien acetylcholine vervangen werd door histamine, eledoisine of prostaglandine $F_{2\alpha}$ en na transmurale electrische stimulatie. Geen relaxatie werd verkregen na verhoging van de tonus van het cavia ileum met bradykinine, kalium- of bariumchloride. Indien, na tonus verhoging, de voorwaarden aanwezig waren voor een bifasische respons van bradykinine, veroorzaakten acetylcholine, histamine, eledoisine, prostaglandine $F_{2\alpha}$ en lysine-vasopressine aléén een contractie, terwijl adrenaline, noradrenaline, oxytocine, calcium- en magnesiumchloride alléén een relaxatie van het ileum gaven. Het bleek dat de door acetylcholine veroorzaakte contractie en de vervolgens door bradykinine geïnduceerde relaxatie negatief gecorreleerd waren. Na herhaalde toevoeging van bradykinine bleek de respons onveranderd. Het ontbreken van tachyphylaxie maakt het waarschijnlijk dat de door bradykinine geïnduceerde relaxatie het resultaat is van een direkte werking van bradykinine op het celmembraan van de gladde spier en niet het gevolg is van een remming van een receptor die na stimulatie een contractie veroorzaakt.

De mogelijkheid dat de door bradykinine veroorzaakte relaxatie van het cavia ileum het gevolg is van het vrijmaken van catecholaminen uit hun opslagplaats in zenuwuiteinden of het gevolg is van een direkte interactie met adrenerge receptoren werd onderzocht m.b.v. enige α- en β-adrenerge remmers. De α-adrenerge remmer piperoxan potentieerde de bradykinine relaxatie terwijl phentolamine, eveneens een α-adrenerge remmer, een remming tot gevolg had. Beide remden echter de acetylcholine contractie maar hadden geen effect op de door adrenaline geïnduceerde relaxatie. De bradykinine-relaxatie van het cavia ileum was

tien maal gevoeliger voor remming door phentolamine dan het duodenum van de rat. De β- adrenerge remmer propranolol, potentieerde de bradykinine relaxatie en remde de acetylcholine contractie. Sotalol, eveneens een β-adrenerge remmer, was minder sterk werkzaam dan propranolol. De door adrenaline veroorzaakte relaxatie kon gedeeltelijk door propranolol worden geremd, terwijl sotalol een nagenoeg 100%-ige remming tot gevolg had. Met een combinatie van phentolamine en propranolol, werd een geringe potentiering van de bradykinine relaxatie en een gedeeltelijke remming van de adrenaline relaxatie verkregen. Hieruit werd geconcludeerd dat de bradykinine-relaxatie niet het resultaat kan zijn van een direkte werking op α- of β- adrenerge receptoren van het cavia ileum. De potentiering van de bradykinine relaxatie door piperoxan en propranolol is waarschijnlijk het gevolg van een indirekte werking, te weten een remming van de acetylcholine contractie. De door phentolamine veroorzaakte vermindering van de bradykinine-relaxatie kan echter niet worden verklaard uit een werking op de acetylcholine contractie. De door adrenaline geïnduceerde relaxatie van het cavia ileum vindt plaats via een stimulatie van β-adrenerge receptoren.

De bradykinine-relaxatie, van het door acetylcholine gecontraheerde ileum wordt minder na verhoging van de acetylcholine concentratie in het orgaanbad. Naar aanleiding hiervan werd nagegaan of andere spasmogenen eveneens de bradykinine-relaxatie konden remmen, en welk mechanisme bij een dergelijke remming betrokken is. Een negatieve correlatie kan worden aangetoond tussen zowel het percentage van de maximale contractie als de concentratie acetylcholine, histamine en eledoisine enerzijds en de bradykinine-relaxatie anderzijds. Regressie analyse toonde aan dat de helling voor acetylcholine steiler was dan die voor eledoisine of histamine. Geen correlatie werd gevonden met angiotensine, hoewel lage niet spasmogene doses, na contractie door acetylcholine, de bradykinine-relaxatie remden. Morphine en atropine remden de angiotensine-contractie, maar potentieerden de bradykinine geïnduceerde relaxatie indien tevens angiotensine aanwezig was. Alléén atropine remde de acetylcholine-contractie en potentieerde de bradykinine-relaxatie in aanwezigheid van acetylcholine. Morphine en atropine hadden beide geen effect op de door bradykinine geïnduceerde remming van zijn eigen respons. De mate van de bradykinine-relaxatie op het cavia ileum bleek eerder afhankelijk van de wijze van stimulatie, dan van de con-

tractie toestand zelf. Tevens bleek de bradykinine-relaxatie gevoelig voor stoffen die een werking hebben op het parasympathische systeem. Zo remde angiotensine de bradykinine-relaxatie via een mechanisme waarbij zijn indirekte parasympathische werking is betrokken. In tegenstelling hiermee bleek de door bradykinine veroorzaakte remming van zijn eigen relaxatie niet van een parasympathische werking afhankelijk te zijn, maar waarschijnlijk het gevolg te zijn van een direkte werking op het membraan van de gladde spier.

Eveneens werd in overweging genomen dat de mate van vermindering van de bradykinine-relaxatie door phentolamine mogelijkerwijs afhankelijk is van de aanwezigheid van verschillende spasmogenen. Het bleek dat phentolamine de bradykinine-relaxatie het sterkst remde na acetylcholine, vervolgens na angiotensine, dan na histamine en eledoisine. Phentolamine gaf altijd een remming van de bradykinine-relaxatie, maar de mate van de remming was afhankelijk van de aard van het spasmogeen. Zo was de remming door phentolamine van de histamine-contractie het grootst, gevolgd door die van acetylcholine en angiotensine. De eledoisine respons werd niet door phentolamine beïnvloed.

De mogelijkheid dat prostaglandinen door bradykinine in het cavia ileum vrijgemaakt worden (*de novo* synthese), welke tot relaxatie zou kunnen leiden, wordt als een mogelijk indirekt mechanisme voor de bradykinine-relaxatie beschouwd. Enige anti-inflammatoire stoffen waarvan bekend is dat zij een remmende werking hebben op de prostaglandine biosynthese óf de bronchoconstrictoire werking van bradykinine op de cavia long blokkeren, zijn getoetst op hun effecten op de bradykinine-relaxatie. Concentraties van indomethacine, natrium salicylaat, phenylbutazon en amidopyrine, waarvan bekend is dat zij de biosynthese van prostaglandinen remmen, hadden geen invloed op de bradykinine-relaxatie. In twee onafhankelijke experimenten met indomethacine bleek dat de tijd die verstreek vóór dat de bradykinine-relaxatie optrad merkbaar werd verlengd. Met hoge concentraties van phenylbutazon werden de bradykinine-relaxatie en acetylcholine-contractie in geringe mate geremd, terwijl hoge concentraties van amido-pyrine de bradykinine-relaxatie potentieerden en de acetylcholine-contractie remden. Hieruit mag worden geconcludeerd dat het onwaarschijnlijk is dat de bradykinine-relaxatie wordt veroorzaakt door biosynthese van prostaglandinen of het mobiliseren van prostaglandine precursors, hoewel de

mogelijkheid niet kan worden uitgesloten dat bradykinine de biosynthese van prostaglandinen stimuleert, en dat gevormde prostaglandinen geen effect op de tonus van het cavia ileum hebben.

Van enkele stoffen is bekend dat zij de bradykinine respons specifiek potentiëren. Bijgevolg werd de mogelijkheid onderzocht van een potentiëring van de bradykinine-relaxatie van het door acetylcholine gecontraheerde ileum. Het effect van cysteine, 2,3-dimercaptopropanol (BAL), α-thiolglycerol en een synthetisch pentapeptide (BPP_{5a}), identiek met één van de bradykinine potentiërende peptiden (BPF) van het gif van *Bothrops jararaca*, werd onderzocht op de door bradykinine geïnduceerde relaxatie en contractie. Deze stoffen potentieerden zowel de bradykinine-relaxatie als de contractie. De bradykinine-relaxatie werd gepotentieerd door concentraties van cysteine en BAL die tien maal lager waren dan die, nodig voor potentiëring van de bradykinine-contractie. Geen verschil werd gevonden tussen de mate van potentiëring van de relaxatie en contractie door α-thiolglycerol en BPP_{5a}. Met hoge concentraties cysteine en BAL werden tegenstrijdige effecten gevonden; met bepaalde concentraties werd een potentiëring en met andere een remming van de bradykinine effecten gevonden. Hoge concentraties cysteine potentieerden, maar BAL remde de acetylcholine-contractie. α-Thiolglycerol en BPP_{5a} hadden geen effect op de acetylcholine-contractie. De mogelijkheid dat verschillende receptoren voor de bradykinine-relaxatie en bradykinine-contractie, van het door acetylcholine gecontraheerde cavia ileum bestaan, wordt besproken.

CURRICULUM VITAE

The author of this thesis was born in 1943 at Woodford Green, England. By 1963 he had successfully gained 10 General Certificate of Education Ordinary level examinations, and further Advanced level examinations in Chemistry, Physics, Pure Mathematics and Zoology. In 1967 after a combination of full and part-time study at North East London Polytechnic, he had succeeded in the Part I and II examinations in Biochemistry of the Institute of Biology (M.I.Biol.). Between 1967-68 and 1969-70 he was a research biochemist at May & Baker Ltd., Dagenham, England, and between 1968-69 a research biochemist at Geigy A.G., Basle, Switzerland. Since 1970 he has been a research associate in the Department of Pharmacology, Medical Faculty, Erasmus University of Rotterdam, The Netherlands.

POST SCRIPT

At the time of writing (September 1973) the following publications have resulted from the work reported in this thesis:-

Hall, D.W.R. and I.L. Bonta, 1972, Neurogenic factors involved in the relaxing effect of bradykinin on the isolated guinea-pig ileum, Arch. Intern. Pharmacodyn. Ther. *197*, 380. (Abstract of a communication presented at the 12th Federation Meeting of the Dutch Medical and Biological Societies, April 22-24th, 1971)

Hall, D.W.R. and I.L. Bonta, 1973a, The biphasic response of the isolated guinea-pig ileum by bradykinin, European J. Pharmacol. *21*, 147.

Hall, D.W.R. and I.L. Bonta, 1973b, Effects of adrenergic blockers on the relaxation of the guinea-pig ileum by bradykinin and adrenaline, European J. Pharmacol. *21*, 139.

Bonta, I.L. and D.W.R. Hall, 1973, Potentiation of the biphasic bradykinin response of the guinea-pig ileum, Brit. J. Pharmacol. Chemother. *In Press*. (Abstract of a communication presented at the British Pharmacological Society, July 12-13th, 1973)