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Localization of Dopamine D_2 Receptor in Rat Spinal Cord Identified with Immunocytochemistry and *In Situ* Hybridization

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Abstract

In the present study the distribution of dopamine D_2 receptors in rat spinal cord was determined by means of immunocytochemistry using an anti-peptide antibody, directed against the putative third intracellular loop of the D_2 receptor and *in situ* hybridization (ISH) using a [^{35}S]UTP labelled anti-sense riboprobe. With the immunocytochemical technique, labelling was confined to neuronal cell bodies and their proximal dendrites. Strongest labelling was present in the parasympathetic area of the sacral cord and in two sexually dimorphic motor nuclei of the lumbosacral cord, the spinal nucleus of the bulbocavernosus and the dorsolateral nucleus. Moderately labelled cells were present in the intermediolateral cell column, the area around the central canal and lamina I of the dorsal horn. Weak labelling was present in the lateral spinal nucleus and laminae VII and VIII of the ventral horn. Except for the two sexually dimorphic motornuclei of the lumbosacral cord labelled motoneurons were not encountered. With the ISH technique radioactive labelling was present in many neurons, indicating that they contained D_2 receptor mRNA. The distribution of these neurons was very similar to the distribution obtained with immunocytochemistry, but with ISH additional labelled cells were detected in laminae III and IV of the dorsal horn, which were never labelled with immunocytochemistry. The present study shows that the D_2 receptor is expressed in specific areas of the rat spinal cord. This distribution provides anatomical support for the involvement of D_2 receptors in modulating nociceptive transmission and autonomic control. Our data further indicate that D_2 receptors are not directly involved in modulating motor functions with the exception, possibly, of some sexual motor functions.

Introduction

Biochemical studies in the seventies (Magnusson, 1973; Commissiong and Neff, 1979) first indicated that dopamine (DA) in the spinal cord acted as a neurotransmitter and not solely as a precursor in the synthesis of other catecholamines. Anatomically, the presence of DA fibres in the spinal cord was first demonstrated by histofluorescence techniques (Björklund and Skagerberg, 1979; Hökfelt *et al.*, 1979; Skagerberg *et al.*, 1982). More recently, immunocytochemical studies using DA-specific antibodies at the light and electron microscopical level revealed that DA fibres and terminals existed in virtually all laminae throughout the spinal cord (Holstege *et al.*, 1990; Shirouzu *et al.*, 1990; Mouchet *et al.*, 1992; Ridet *et al.*, 1992). DA immunoreactivity was most pronounced in the dorsal horn, in the area around the central canal, in the sympathetic intermediolateral cell column (IML) and in the motoneuronal cell groups. Retrograde tracing techniques combined with histofluorescence (Björklund and Skagerberg, 1979) or immunohistochemistry of catecholamine-synthesizing enzymes, like tyrosine hydroxylase (Hökfelt *et al.*, 1979; Skagerberg *et al.*, 1988) and aromatic L-amino decarboxylase (Skagerberg *et al.*, 1988), indicated that the spinal DA innervation originated exclusively from the A-11 catecholamine cell group, which is situated in the periventricular posterior hypothalamus. The paraventricular nucleus has also been implicated as a minor source for the spinal DA innervation (Swanson *et al.*, 1981). Thus, the existence of an extensive DA innervation of the spinal cord, mainly originating from the A-11 group, is now well established.

Over the years, biochemical, pharmacological and electrophysiological studies (Barasi and Roberts, 1977; Coote *et al.*, 1981; Fleetwood-Walker *et al.*, 1988) provided evidence for specific effects of DA within the spinal cord which were mediated by DA receptors. Presently, DA receptors are subdivided in two receptor families based on pharmacological and biochemical criteria (for reviews see Civelli *et al.*, 1991; Gingrich and Caron, 1993). These receptor families include D_1-like receptors, which are stimulatory coupled to adenylate cyclase and D_2-like receptors, which are either inhibitory or not coupled to this enzyme. Subtypes belonging to the two DA receptor families have been cloned and sequenced: D_1-like receptors include the D_1 and D_5 DA receptors, whereas D_2-like receptors include the D_2, D_3 and D_4 dopamine receptors. By alternate splicing of the D_2

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receptor gene two isoforms (D_{2short} and D_{2long}) are generated, which differ by a splice insert of 29 amino acids in the putative third intracellular loop of the D_2 receptor. This insert is present in the long isoform, but is lacking in the short isoform.

The specific distribution of DA receptors in the central nervous system has been studied with receptor ligand binding, ISH and more recently immunocytochemistry. So far, the few studies that were focused on the distribution of DA receptors in the spinal cord were mainly based on receptor ligand binding. Binding studies on homogenized rat spinal cord first suggested the presence of DA D_1 receptors in the spinal cord (Bhargava and Gulati, 1990) especially in its ventral part (Dubois *et al.*, 1986). In contrast, D_2 receptor binding sites were found mainly in the dorsal horn of the spinal cord (Demenge *et al.*, 1980; Dubois *et al.*, 1986; Bouthenet *et al.*, 1987), especially laminae II and III and near the central canal (Scatton *et al.*, 1984). More recently, an extensive binding study on the D_2 receptor with the highly selective compound YM-09151-2 confirmed and extended these findings (Yokoyama *et al.*, 1994) by showing additional binding in the IML and motoneuronal cell groups of the ventral horn. The presence of D_2 receptor mRNA in the spinal grey matter has also been reported, but no anatomical details were provided (Bouthenet *et al.*, 1991). However, the interpretation of the results obtained with the ligand binding technique is often difficult because the selectivity of the ligands and the anatomical resolution are limited. The immunocytochemical method provides a better anatomical resolution and may also be a more accurate tool to distinguish between different DA receptors and their subtypes than the current ligand binding techniques. Only after the discovery of the sequence of the D_2 receptor (Bunzow *et al.*, 1988) it became possible to prepare specific antibodies, using fusion proteins or synthetic peptides corresponding to a specific part of the D_2 receptor. These peptide fragments have been used successfully for raising polyclonal antibodies against the D_2 receptor (McVittie *et al.*, 1991; Ariano *et al.*, 1993; Boundy *et al.*, 1993a, b; Chazot *et al.*, 1993; Sesack *et al.*, 1994; Smiley *et al.*, 1994) and other DA receptors, including the D_1 and D_3 receptor (Ariano and Sibley, 1994; Smiley *et al.*, 1994). Some of these immunocytochemical studies also reported the presence of DA receptors in the spinal cord (Ariano *et al.*, 1993; Smiley *et al.*, 1994); however, no anatomical details were provided. Taken together, the available data, mostly based on receptor ligand binding, suggest that DA D_1 receptors are predominantly located in the ventral horn, while D_2 receptors are mainly localized in the dorsal horn, but may also be found in motoneuronal cell groups of the ventral horn.

In the present study we have used a polyclonal antipeptide antibody (Plug *et al.*, 1992) for mapping the D_2 receptor in rat spinal cord. In addition we have applied the ISH technique to verify and extend the data obtained with immunocytochemistry, using an anti-sense [^{35}S]UTP labelled riboprobe for detection of D_2 receptor mRNA.

Materials and methods

Preparation and characterization of the D_2 receptor antibody

Preparation and characterization of the antibody used in the present study has been described previously (Plug *et al.*, 1992). Briefly, a polyclonal antiserum (pAb2) was obtained from rabbits after injecting a synthetic peptide coupled to keyhole limpet haemocyanin. The peptide was derived from an amino acid sequence (amino acids 301–315) in the third intracellular loop of the rat D_2 receptor, which is present in both the short and long isoform. For the purpose of the present study two different affinity gels were used to obtain purified antibodies from this antiserum: (i) Protein A–Sepharose 6MB (Pharmacia) and (ii) synthetic peptide coupled directly to CNBr-activated Sepharose 4B. In each case 500 µl antiserum was incubated with 500 µl of the corresponding gel in a total volume of 5 ml 10 mM Tris–HCl (pH 7.4), 250 mM NaCl. After 16 h at 4°C the gel suspension was transferred to a 3 ml disposable column and washed extensively with Tris–HCl buffer. Specific antibodies were eluted with 3 column vol of 0.1 M glycine-chloride (pH 2.5) and neutralized immediately by collecting them in 0.3 column vol 1 M Tris–HCl (pH 8.0), followed by addition of bovine serum albumin solution to a final concentration of 1 mg/ml. The collected purified antisera were stored frozen at –80°C until use. The antibodies obtained with the protein A-sepharose 6MB gel gave the most intense staining in all regions of the spinal cord and antibodies obtained with this method were mainly used for the purpose of this study.

Immunocytochemistry

Adult, male Wistar rats were deeply anaesthetized with sodium pentobarbital and perfused transcardially with 50 ml saline, followed by 500–750 ml 4% (wt/vol) freshly made paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). In some cases up to 0.05–0.2% (vol/vol) glutaraldehyde was added to the fixative and best results were obtained with 0.1% glutaraldehyde. The fixation liquid was followed by 150 ml of 10–15% (wt/vol) sucrose in phosphate buffer. After perfusion the brains and spinal cords were removed and stored overnight at 4°C in phosphate buffer containing 20–25% (wt/vol) sucrose. The next day 30 µm frozen sections were cut from all spinal segments and selected parts of the brain and collected in 0.1 M Tris-buffered saline (pH 8.6). All subsequent procedures were performed on free-floating sections at room temperature except where specified otherwise. After each step sections were thoroughly rinsed with Tris-buffered saline. Sections were first incubated with a solution of 5% (vol/vol) normal goat serum (Gibco) in Tris buffered saline containing 0.3% (vol/vol) Triton X-100 for 90 min to reduce background staining. Next, sections were incubated for 24–48 h at 4°C with polyclonal D_2 receptor antipeptide antibody (1:500) followed by biotinylated goat–anti-rabbit (1:200, 120 min) and the ABC procedure (ABC Elite, Vector). The peroxidase complex was visualized with 0.05% (wt/vol) 3,3′-diaminobenzidine (Sigma) in 0.05 M Tris–HCl solution (pH 7.6) in the presence of 0.01% (vol/vol) hydrogen peroxide, which yields a brown precipitate. In some cases 0.01% (wt/vol) nickel ammonium sulphate solution was added, which resulted in a dark blue precipitate. After completion of the immunocytochemical procedure sections were mounted on to glass slides with gelatin-chrome alum adhesive and allowed to dry at room temperature. Next, sections were dehydrated in graded ethanol baths, transferred to xylene and cover-slipped with Permount. Some slides were counterstained with cresyl violet. Sections were examined light microscopically with bright-field illumination.

Immunocytochemical controls

Controls for the immunocytochemical methods included: (i) substitution of the antipeptide antiserum with pre-immune serum; (ii) substitution of the primary antiserum with saline; and (iii) pre-adsorption of the antiserum with an excess of its peptide antigen (1 mg/ml antiserum) prior to incubation. Omission of the primary antibody or substitution of pre-immune serum for the antipeptide antibody resulted in clean sections without staining. Pre-adsorption of the antiserum with an excess of its peptide antigen prior to incubation prevented neuronal staining, except for some white matter glia, which is, therefore, considered as background staining. Adding

glutaraldehyde to the perfusion fixative resulted in reduction of background staining, including the white matter glia, while the labelling in the areas described remained, albeit less intense.

In the brain D_2 receptor immunoreactivity was found in many areas, among which the cerebral cortex, striatum, substantia nigra pars compacta, hippocampus, ventral tegmental area, superficial layers of superior colliculus and hypothalamus. The labelling obtained in brain was very similar to previous anatomical studies on the localization of D_2 receptor using ISH (Weiner *et al.*, 1991; Brouwer *et al.*, 1992), receptor ligand binding (Dubois *et al.*, 1986; Bouthenet *et al.*, 1987; Yokoyama *et al.*, 1994) and immunocytochemistry (Brock *et al.*, 1992; Ariano *et al.*, 1993; Levey *et al.*, 1993). We therefore concluded that the immunocytochemical labelling of neuronal elements obtained in the present study represented D_2 receptor protein.

In situ hybridization

Rats were decapitated and their spinal cords were quickly removed and frozen on dry ice. Cryostat sections (10 μm) from thoracic, lumbar and sacral levels of the spinal cord were made and collected on gelatin-coated slides, air dried at room temperature and fixed with 4% (wt/vol) paraformaldehyde solution in 0.05 M phosphate-buffered saline (pH 7.4). After three rinses in phosphate-buffered saline, sections were treated with acetic anhydride, dehydrated in graded ethanol baths, followed by two successive chloroform baths for 10 min each. Next, slides were allowed to air dry and were stored at −70°C until further processing.

Sections were hybridized with [^{35}S]UTP labelled anti-sense riboprobe for D_2 receptor as described (Jongen-Rêlo *et al.*, 1994). After hybridization and stringency washing steps the sections were air dried, dipped into nuclear photographic emulsion (Kodak NTB-2) and stored in the dark (4°C). After 12–16 weeks exposure the sections were developed, fixed and counterstained with cresyl violet. The distribution of labelled neurons was analysed light microscopically with dark-field illumination.

Specificity controls for the ISH included hybridization with 'sense' probe and tissue treatment with RNase prior to anti-sense probe hybridization. Both methods resulted in absence of labelling and confirmed the specificity of the hybridization signal generated by the radioactively labelled riboprobe.

Results

Immunocytochemistry

In all positive-staining regions examined in the central nervous system the DAB reaction product, indicating the presence of D_2 receptor protein, was mostly associated with neuronal cell bodies and their proximal dendrites, but in some cases the dendritic processes could be followed for some distance within a section. Application of the antibody on spinal cord sections resulted in a distinct pattern of labelled neurons. In comparison with most brain areas, labelled neurons in the spinal cord were few and weakly labelled. However, some strongly labelled neurons were also observed (see below).

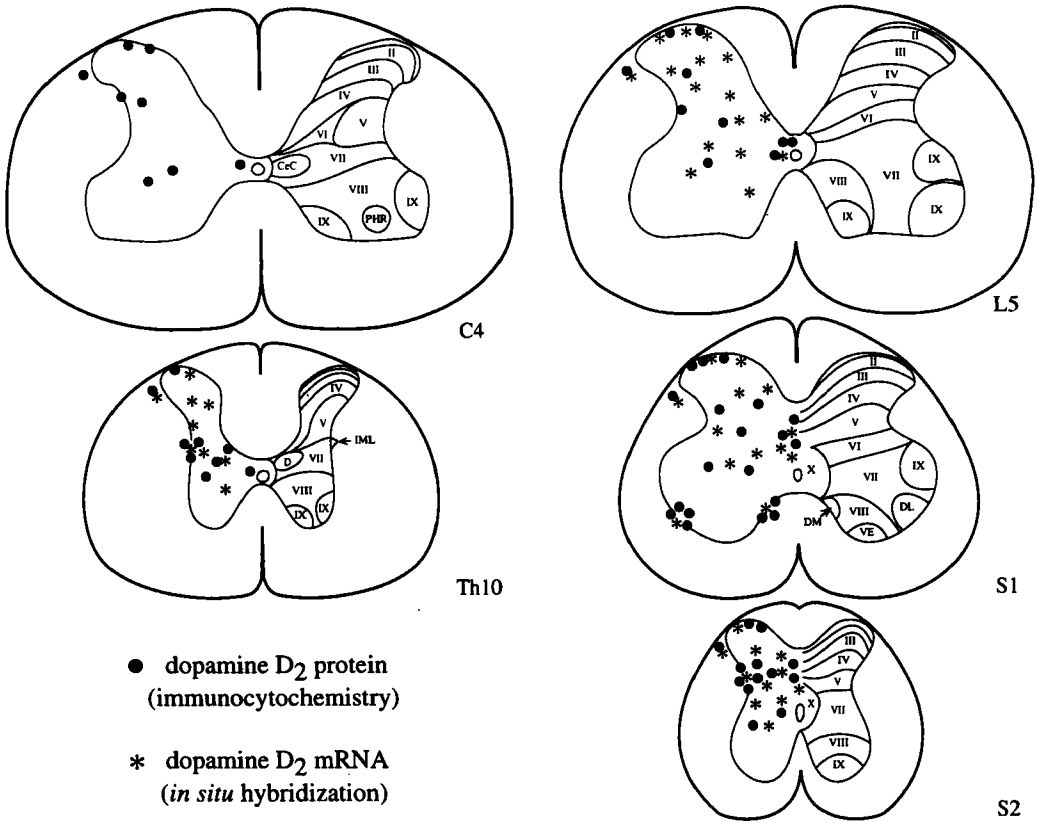

FIG. 1. Schematic representation showing the distribution of neurons immunoreactive for dopamine D_2 receptor protein (filled dots; 1 dot = 1–3 cells) or radioactively labelled for D_2 receptor mRNA (stars; 1 star = 3–5 cells), as revealed by immunocytochemistry and *in situ* hybridization. Note that no stars were drawn in the first segment (C4), because high cervical levels were not processed for *in situ* hybridization.

At all levels of the spinal cord labelling was found exclusively in cell bodies and their proximal dendrites. The immunoreactivity was virtually absent from other neuronal processes, like axons, small dendrites nor was there any indication for localization of immunoreactivity in terminals. The labelled neurons were located in specific areas throughout the spinal cord, including the superficial dorsal horn, the region surrounding the central canal, the sympathetic IML and the sacral parasympathetic area and, to a lesser extent, in the ventral horn (Fig. 1).

In the dorsal horn densely labelled neurons were present in lamina I (Fig. 2A). In every section examined, an average of one to three labelled cells were observed. They were found at all levels of the spinal cord and were distributed both medially and laterally within lamina I. In the lateral spinal nucleus, located in the lateral funiculus of the spinal cord, moderately labelled cells were occasionally present (Fig. 2B). At high cervical levels no labelled cells were seen in the lateral cervical nucleus, although it was sometimes difficult to delineate its boundary with the lateral spinal nucleus. In each spinal cord section, from high cervical to low sacral levels, on average one or two cells were labelled in the lateral spinal nucleus. No labelled cells were observed in lamina II and III of the dorsal horn. In lamina IV and V some scattered weakly labelled cells were present and occasionally a moderately labelled cell was encountered. In general, when going from lamina IV to laminae V and VI more labelled cells

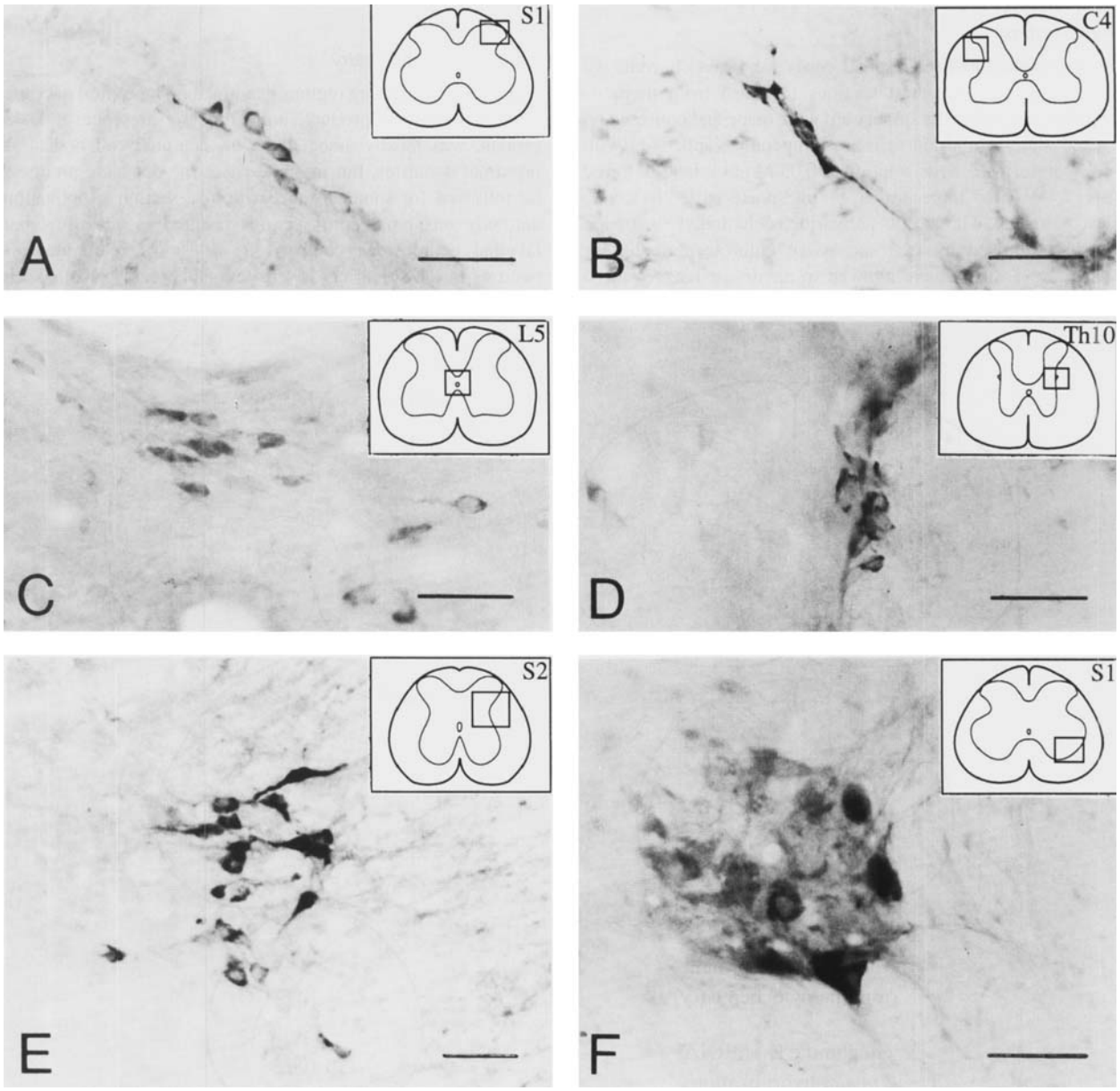

Fig. 2. (A–F) Light micrograph of dopamine D_2 receptor protein immunoreactive cells in different areas of the rat spinal cord as indicated schematically in each inset. (A) Lamina I; (B) lateral spinal nucleus of the dorsal horn; (C) area around the central canal; (D) preganglionic cells of the intermediolateral cell column of the thoracic cord; (E) parasympathetic area of the sacral cord; (F) sexually dimorphic motoneurons in the dorsolateral nucleus of the ventral lumbosacral cord. Scale bar = 50 μm.

were observed and the labelling became more intense, although they were still not strongly labelled. In the area around the central canal moderately and weakly labelled cells were present at all levels of the spinal cord, but they were somewhat more intense at the lumbar level (Fig. 2C). The labelled cells were scattered around the central canal and clusters of moderately labelled cells were present in the area immediately dorsal of the central canal.

In the IML of the spinal cord, at thoracic and high lumbar levels, clusters of five to six moderately labelled cells were present (Fig. 2D) in many, but not all sections. The labelling extended into the proximal dendrites radiating towards the lateral funiculus. A high proportion of the neurons of the IML were immunoreactive. Additional labelled cells were located in related areas towards the central canal (intercalated nuclei), but these cells were less intensely labelled.

Strongest labelling within the spinal cord was found in cells of the parasympathetic area of the sacral cord (Fig. 2E) and a high proportion of the neurons in this area were immunoreactive. The labelled cells showed a dense staining of their cell bodies and proximal dendrites. They were arranged in clusters of four to five cells, with their dendrites radiating towards the lateral funiculus. In addition to these strongly labelled cells also several moderately labelled cells were present in this area.

In the ventral horn, a few moderately and weakly labelled cells were present in laminae VII and VIII. In general, when going more ventrally, staining of labelled cells became faint. Labelled motoneurons were found only in the lumbosacral spinal nucleus of the bulbocavernosus (SNB) and dorsolateral nucleus (DLN) (Fig. 2F), which innervate different target muscles: the DLN innervates the external urethral sphincter, while the SNB innervates the external anal sphincter and the bulbocavernosus. Motoneurons of the SNB and DLN were labelled equally strong and their proximal dendrites were also densely labelled. A light staining of the neuropil surrounding the SNB and DLN was observed, probably due to faint labelling of small dendrites, which are present in longitudinally oriented bundles. Labelled motoneurons were never observed at other levels of the spinal cord.

In situ hybridization

The presence of D_2 receptor mRNA was detected with ISH. Radioactively labelled riboprobe was hybridized to D_2 receptor mRNA, which resulted in a distinct accumulation of silver grains over many cell bodies (Fig. 3A, B).

In general, labelling of neuronal cell bodies was found in the same areas of the spinal grey matter as described for immunocytochemistry (Figs 1 and 3A), but in many of these areas a larger number of neurons were labelled with the ISH technique. Labelled cells were present in lamina I of the superficial dorsal horn and in the lateral spinal nucleus, while no labelling was seen in lamina II. Some labelled cells were detected in lamina III and IV of the dorsal horn (Fig. 3A), which is in contrast with the immunocytochemical findings. In the base of the dorsal horn labelled cells were scattered throughout lamina V and VI. In the area around the central canal labelled cells were present in clusters, predominantly located in the area immediately dorsal of the central canal. In the IML of the thoracic cord clusters of five to six labelled cells were observed and additional labelled cells were present in the intercalated nuclei towards the central canal (Fig. 3B). In the sacral parasympathetic area labelled cells were also present. Furthermore, in the ventral horn some labelled cells were scattered throughout lamina VII and VIII. In the motoneuronal cell groups, however, no labelling was detected except for some labelled cells in the lumbosacral SNB and DLN.

Discussion

In the present study, for the first time the regional distribution of D_2 receptor protein and mRNA was identified in rat spinal cord using immunocytochemistry and ISH. The results showed a characteristic

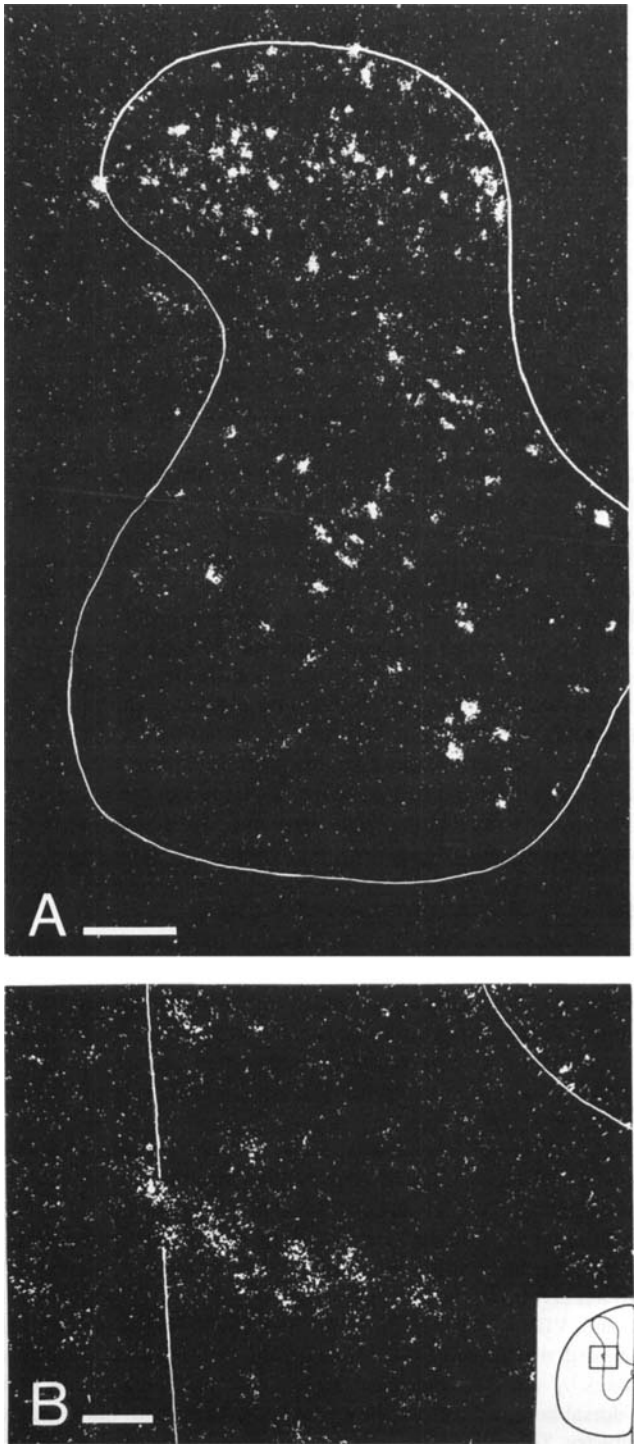

Fig. 3. Darkfield microautoradiographs of selected parts of the rat spinal cord showing accumulation of silver grains over neurons containing detecting dopamine D_2 receptor mRNA hybridized to ^{35}S-labelled riboprobe. (A) Lumbar cord; (B) lateral horn of thoracic cord. Scale bar = 200 μm and 50 μm respectively.

distribution of D_2 receptors throughout the spinal cord, providing anatomical support for the involvement of D_2 receptors in the effects of DA in the spinal cord.

Technical considerations

The observation that D_2 receptor immunoreactivity was located mainly within the somata and proximal dendrites of labelled neurons is in agreement with other studies using different D_2 receptor antibodies (Brock *et al.*, 1992; Ariano *et al.*, 1993). A subsequent study (Fisher *et al.*, 1994) at the electron microscopical level showed that in neostriatal neurons the D_2 receptor immunoreactivity was uniformly present throughout the somatic cytoplasm and became less strong towards the periphery. The immunoreactivity was not associated with any cellular organelle. Some axon terminals contained traces of immunoreactivity, but labelled postsynaptic densities were not described. These findings may suggest that the antibody only recognized D_2 receptor protein before its incorporation as a functional receptor into the neuronal membrane or during its degradation. If this also holds true for the antibody used in the present study, it would explain that only cell somata and proximal dendrites were stained. On the other hand, if the antibody would recognize both functional and non-functional receptors, our results would indicate that the highest proportion of D_2 receptor protein is located within the cell body and proximal dendrites while there are only minute amounts of functional receptors in more distal dendrites.

The long exposure times (up to 3 months) that were needed to visualize the radioactively labelled riboprobe indicated that the hybridization signal was relatively low. Similarly, with immunocytochemistry it was noted that, compared with other parts of the central nervous system, the spinal cord contained relatively few cells that were immunoreactive for the D_2 receptor and that most of them were not strongly labelled. Thus, it seems likely that in comparison with other brain areas the level of D_2 receptors in rat spinal cord is low both at the protein and mRNA level. Owing to these low expression levels the total number of cells expressing D_2 receptors may have been underestimated.

Distribution of D_2 receptors in spinal cord

The results of the present study showed that the D_2 receptor is expressed in several areas of the rat spinal cord. In all spinal segments investigated with immunocytochemistry or ISH, the distribution obtained with each technique was very similar, i.e. all areas with neurons immunoreactive for D_2 receptor protein also showed neurons expressing D_2 receptor mRNA, although more labelled cells were detected with ISH.

With immunocytochemistry, strongest labelling was present in neuronal cell bodies located in the sacral parasympathetic area and in the lumbosacral SNB and DLN. Moderately labelled cells were present in the IML, in the area around the central canal and in lamina I of the superficial dorsal horn, while weak labelling was present in neuronal cell bodies located in the lateral spinal nucleus and lamina VII and VIII of the ventral horn. The results obtained with ISH confirmed this distribution and extended the immunocytochemical findings by showing additional labelled cells in lamina III and IV of the dorsal horn, an area which was never labelled with immunocytochemistry. This difference may be explained by the fact that in this region the concentration of D_2 receptor protein may be very low and, therefore, undetectable with immunocytochemistry. As a consequence our findings indicate that for detection of D_2 receptor expression in the spinal cord the ISH technique may be a more sensitive technique than the immunocytochemistry technique. Finally, it should be noted that the identification of the spinal laminae and nuclei was based on cytological and topographical criteria. As a consequence, in the sacral parasympathetic area there is no direct evidence that neurons expressing the D_2 receptor actually represent parasympathetic preganglionic neurons, as strongly suggested by their location. This can only be ascertained by using double-labelling studies which combine D_2 receptor localization with the appropriate technique for identifying specific neurons.

Previous studies on the regional distribution of D_2 receptors in spinal cord all used the receptor ligand binding technique. Ligand binding was reported in the dorsal horn (Scatton *et al.*, 1984; Dubois *et al.*, 1986; Bouthenet *et al.*, 1987; Yokoyama *et al.*, 1994), the area around the central canal (Scatton *et al.*, 1984; Yokoyama *et al.*, 1994), the IML and in motoneurons (Yokoyama *et al.*, 1994). Our findings are in agreement with these binding studies, but differed from the reported binding in the substantia gelatinosa and in the motoneuronal cell groups, areas which showed neither immunoreactivity nor hybridization signal. However, it must be kept in mind that the binding pattern may differ from the cellular distribution obtained with ISH or immunocytochemistry, because the functional D_2 receptors, i.e. the binding sites, may be located on dendrites of neurons which have their cell somata in another lamina of the spinal cord. With respect to the binding found in the substantia gelatinosa our findings indicate that the parent cell bodies of these D_2 receptor binding sites are located outside the substantia gelatinosa of the dorsal horn (lamina II). On the other hand, the ligands may also bind to other receptors of the D_2 receptor family, due to a lack of pharmacological selectivity which is inherent to the receptor ligand binding technique. This may be true especially for the reported ligand binding in spinal motoneurons (Yokoyama *et al.*, 1994), because neither binding studies nor *in situ* hybridization studies have described the presence of D_2 receptors in motoneurons. Moreover, we never observed D_2 receptor labelling in motoneurons outside the lumbosacral SNB and DLN. Therefore, we consider the presence D_2 receptors in spinal motoneurons, except the SNB and DLN, most unlikely.

Functional aspects

The distribution of D_2 receptors in rat spinal cord, as demonstrated with immunocytochemistry and ISH, suggests that D_2 receptors may play a part in the transmission of sensory information and autonomic processing. Our results also indicate that D_2 receptors are not directly involved in modulation of motoneurons, with the exception of motoneurons in the SNB and DLN of the lumbosacral cord.

Several studies indicate that spinal DA and, more specifically, D_2 receptors play an inhibitory part in the transmission of nociceptive stimuli. It has been reported that intrathecally administered D_2 receptor agonists produced a reversible reduction of thalamic nociceptive responses to noxious stimuli (Clatworthy and Barasi, 1987). Furthermore, focal electrical stimulation in the region of the A-11 cell group selectively suppressed nociceptive responses of spinal multireceptive neurons (Fleetwood-Walker *et al.*, 1988). This inhibition was rapidly reversed by ionophoresis of the D_2 receptor antagonist sulpiride in the superficial and deeper dorsal horn. Similarly, D_2 receptor antagonists antagonized the antinociceptive effects induced by intrathecally applied DA (Liu *et al.*, 1992). In contrast, neither the D_1 receptor agonist SKF38393 nor D_1 receptor antagonist SCH23390 showed any effect on the DA-induced antinociception (Barasi *et al.*, 1987; Liu *et al.*, 1992). These findings suggest that the DA-induced antinociceptive effects are mediated via spinal D_2 receptors located in the superficial and deeper dorsal horn. This is in good agreement

with the regional distribution of D_2 receptors in the dorsal horn as described in the present study.

The presence of D_2 receptors in the IML of the thoracic and high lumbar cord is in agreement with its dense DA innervation (Yoshida and Tanaka, 1988; Holstege *et al.*, 1990; Shirouzu *et al.*, 1990; Mouchet *et al.*, 1992; Ridet *et al.*, 1992). Apomorphine and other, more selective, D_2 receptor agonists administered intrathecally down-regulated autonomic functions, resulting in hypotension and bradycardia (Petitjean *et al.*, 1984; Clatworthy and Barasi, 1987; Pellissier and Demenge, 1991; Lahlou and Demenge, 1993). Our findings suggest that the inhibition by DA of preganglionic cells of the IML is mediated by D_2 receptors.

D_2 receptors were found to be most prominent in neurons of the sacral parasympathetic area and the lumbosacral SNB and DLN, which innervate anatomically distinct perineal muscles that are involved in functionally distinct copulatory reflexes. Previous studies have shown that DA agonists inhibit specific sexual motor functions like penile reflexes (Stefanick *et al.*, 1982; Pehek *et al.*, 1989). Our results provide some anatomical support for the involvement of D_2 receptors located in the lumbosacral cord in the regulation of sexual motor behaviour at the spinal level.

There is also some evidence favouring the idea that DA is involved in the control of spinal motor functions. Although DA is present in the ventral cord, including motoneuronal cell groups, our results indicate that D_2 receptors are not directly involved in modulating motoneuron activity. The reported effects of DA on somatic motor functions at the spinal level are contradictory. DA receptor mediated depression of the spinal monosynaptic transmission has been reported (Carp and Anderson, 1982; Ono and Fukuda, 1984) and denied (Kamijo *et al.*, 1993). Recently, it has been reported that DA enhances glutamate-activated currents in cultured chick embryo spinal motoneurons. The enhancement was diminished by the D_1 receptor antagonist SCH23390 and mimicked by the (partial) D_1 receptor agonist SKF38393 (Smith *et al.*, 1995). These findings suggest that in the motoneuronal cell groups the presence of DA and, more particularly, the absence of D_2 receptor labelling in motoneurons may indicate that other DA receptors, like D_1 receptors, are more important in this part of the spinal cord.

It may be concluded that the D_2 receptors are expressed in specific areas of the rat spinal cord. Although the level of D_2 receptor in spinal cord may be much lower than in the brain, several physiological studies indicate that activation of spinal D_2 receptors has important functional consequences. Their distribution provides anatomical support for the involvement of spinal D_2 receptors in the modulation of nociceptive transmission and autonomic control. Our data further indicate that spinal D_2 receptors are not directly involved in modulating motor functions at the motoneuronal level with the possible exception of some sexual motor functions. However, it should be mentioned that motor functions may be modulated indirectly via D_2 receptors located in the dorsal horn or at supraspinal levels. In order to elucidate further the role of DA in modulating spinal cord functioning further investigation of various types of DA receptors in the spinal cord needs to be undertaken.

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Abbreviations

DA	dopamine(ergic)
DLN	dorsolateral nucleus
IML	intermediolateral cell column
SNB	spinal nucleus of the bulbocavernosus

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