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Changes in cholinergic and opioid receptors in the rat spinal cord, dorsal root and sciatic nerve after ventral and dorsal root lesion

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Summary. Changes in the distribution of ^{3}H-quinuclidinylbenzilate (^{3}H-QNB), ^{3}H-acetylcholine (^{3}H-ACh) and ^{3}H-alpha-bungarotoxin (alpha-BTx) binding sites were studied with the use of quantitative in vitro autoradiography in the L4–L6 segments of rats 7 days after ventral L4–L6-rhizotomies and 24 hours after ligation of the dorsal roots L4–L6. The changes in the binding sites of these ligands and of ^{3}H-etorphine binding sites were also studied in the dorsal roots of the rats operated with dorsal root ligation and in the sciatic nerves (around a ligature) in the rats operated with ventral rhizotomy. After ventral rhizotomy ^{3}H-QNB binding sites in the ipsilateral motor neuron area were decreased by about 25% from 100 ± 5 to 73 ± 5 fmol/mg wet weight. After dorsal root ligation ^{3}H-QNB binding sites in the ipsilateral posterior horn were reduced by about 30% from 91 ± 5 to 64 ± 7 fmol/mg wet weight. No significant changes in the binding of the other cholinergic ligands in the spinal cords were observed after the operations. In the dorsal root ^{3}H-alpha-Btx and ^{3}H-etorphine binding sites were higher on the distal side of the ligation (3.5 ± 0.8 and 14 ± 4 fmol/mg wet weight, respectively) than on the proximal side (0.7 ± 0.5 and 2.4 ± 1.2 fmol/mg wet weight, respectively).The same level of ^{3}H-ACh (total, muscarinic and nicotinic) binding was observed on both sides of the ligation. In the sciatic nerve ^{3}H-QNB and total, muscarinic and nicotinic ACh binding sites were higher on the proximal side of the ligation than on the distal side. Except for a small emergence of muscarinic-ACh binding distally to the ligation there were no changes in the number of binding sites in the sciatic nerve after the ventral rhizotomy.

Muscarinic antagonist binding sites are probably located on the perikarya of the motor neurons and presynaptically on the primary afferents in the posterior horn and in the dorsal root. Cholinergic agonist binding sites in the spinal cord seem less sensitive to axonal damage than antagonist binding sites. Cho-

linergic and opioid receptors in peripheral nerves are transported in both anterograde and retrograde directions and their origin seems to be the dorsal root ganglion.

Keywords: Cholinergic receptors, opioid receptors, spinal cord, dorsal root, peripheral nerve.

Introduction

Axonal transport in peripheral nerves has been shown for cholinergic [^{3}H-quinuclidinylbenzilate (QNB)] (Wamsley et al., 1981; Gulya and Kasa, 1983; Laduron, 1984; Zarbin et al., 1982), ^{3}H-alpha-bungarotoxin (alpha-Btx) (Ninkovic and Hunt, 1983) and for opioid (^{3}H-etorphine) (Young et al., 1980; Laduron et al., 1984; Laduron and Larssen, 1986) binding sites. As there is no synthesis of protein in nerve terminals, the origin of these binding sites must be in the perikarya in the spinal cord, the spinal root ganglion or the sympathetic ganglion. Information in this field is, however, sparse and only based on non- and semiquantitative autoradiographic studies (Wamsley et al., 1981; Gulya and Kasa, 1983; Ninkovic and Hunt, 1983).

The aim of this investigation was to determine the changes in 1) cholinergic binding sites in the spinal cord and peripheral nerve after ventral rhizotomy; 2) cholinergic binding sites in the spinal cord and dorsal root after dorsal root ligation; 3) opioid binding sites in peripheral nerves after ventral rhizotomy and in the dorsal root after dorsal root ligation, thereby elucidating the transport and origin of these binding sites.

Materials and methods

Nine Sprague-Dawley rats weighing 300–400 g were used. After intraperitoneal chloral hydrate anaesthesia (450 mg/kg) the rats were mounted in a Kopf stereotaxic frame. In 5 of the rats, the ventral root of L4–L6 (the sciatic nerve is derived from these roots) (Hebel and Stromberg, 1976) was cut on the left side under a Zeiss operation microscope after appropriate laminectomies with dura splitting in the midline. Great care was taken to avoid vascular damage. Seven days after this operation the rats were again anaesthetized and a ligature was applied to the sciatic nerve in the mid-high region on both sides. Twenty-four hours later the rats were exsanguinated under deep anaesthesia. The segments L4–L6 and 16 mm of the sciatic nerve at the site of the ligation were dissected out. In 4 rats, a ligation was applied to the dorsal roots of L4–L6 at the level of the corresponding intervertebral foramina on the left side. These operations were also performed under a Zeiss operation microscope and the same precautions as above were taken. Twenty-four hours after the operation the rats were killed as described above and the segments L4–L6 and the corresponding dorsal roots were dissected out.

The tissue samples were immediately frozen between two gold plates in liquid nitrogen and thereafter sectioned in a cryostat (Lab-Tek Products, Naperville, USA). The cryosections (10 µm) of the tissues were thaw mounted on chrome alum/gelatin coated slides and stored at −20 °C until incubated.

For ^{3}H-QNB autoradiography sections were incubated for one hour at 25 °C in 0.05 M Na-K phosphate buffer (pH 7.4) containing 1 nM ^{3}H-QNB (40.2 Ci/mmol, NEN, UK). In

parallel sections were incubated in the same medium with the addition of 1×10^{-6} M atropine. The sections were rinsed for 10 min in ligand-free buffer.

For ^{3}H-ACh autoradiography sections were preincubated for 10 min at +4 °C in A) 50 mM tris-HCl buffer (pH 7.4 at 25 °C) containing 1 mM $MgCl_2$, 120 mM NaCl, 5 mM KCl and 2 mM $CaCl_2$, B) the same buffer containing 1.5 μM atropine sulphate, C) the same buffer containing 1 mM nicotine tartrate, or D) the same buffer containing 100 μM carbacholine and 1.5 μM atropine. A second 10 min preincubation was performed in buffer A containing 100 μM paraoxone (Pestanal, Sigma, USA) a cholinesterase inhibitor. After drying at +4 °C for 5–10 min, the sections were incubated for 40 min in solutions of the same composition as described above (A: total, B: nicotinic, C: muscarinic, D: non-specific binding) containing 20 nM ^{3}H-ACh (86 Ci/mmol, Amersham, UK) and 100 μM paraoxone. The sections were rinsed twice for 5 min in preincubation buffer at +4 °C without paroxone and dipped in distilled water.

For ^{3}H-alpha-Btx autoradiography sections were incubated for one hour at 25 °C in 0.1 M Na-K-phosphate buffer (pH 7.4) containing 3 nM ^{3}H-alpha-Btx (93 Ci/mmol, Amersham, UK). In parallel sections were incubated in the same medium with the addition of 1×10^{-3} M nicotine tartrate. The sections were rinsed 3 times for 10 min each time in ligand-free buffer.

For ^{3}H-etorphine the autoradiography sections were incubated for 20 min at 25 °C in 170 mM tris-HCl buffer (pH 7.4) containing 2 nM ^{3}H-etorphine (33 Ci/mmol, Amersham, UK). In parallel sections were incubated in the same medium with the addition of 1×10^{-6} M levorphanol. The sections were rinsed twice for 5 min in ligand-free buffer at +4 °C.

Atropine sulphate was obtained from Apoteksbolaget, Sweden, nicotine from Leo AB, Sweden and levorphanol from Hoffman La Roche, Basel, Switzerland. All sections were dried and stored frozen with a dessicant for 1–2 weeks. The slides were then covered with film (Hyperfilm, Amersham, UK) and exposed at 4 °C for: QNB 5 weeks, ACh 12 weeks, a-Btx 14 weeks, etorphine 9 weeks and then developed and fixed.

Optical density in the motor neuron area (lamina IX), in the posterior horn (laminae II–III), and in the dorsal root and sciatic nerve proximal and distal to the ligation (2 mm on each side of the ligation was analysed) was measured by an Imtec scanner and image display system (Uppsala, Sweden). The mean grey values obtained were converted into molar concentrations of bound ligand with the use of autoradiographic microscales (Amersham, UK). Non-specific binding was defined as the labeling of tritiated ligand in the presence of non-radioactive ligand.

Results

Ventral rhizotomy

The QNB binding in the motor neuron area on the ipsilateral side of the lesion was significantly decreased compared to the control side (Fig. 2). There was a tendency to decreased total and muscarinic ACh binding on the operated side compared to the control side, but the difference was not significant (Fig. 3). No difference between the two sides was observed for nicotinic ACh and alpha-Btx binding.

In the dorsal horn there was a tendency to decreased binding of all cholinergic ligands on the operated side, but these differences were not significant (Figs. 2 and 3).

In the sciatic nerve the accumulations of QNB and total, muscarinic and

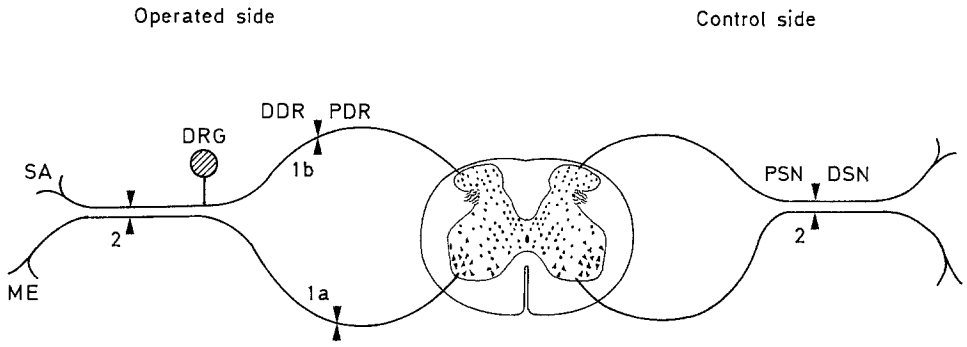

Fig. 1. Schematic drawing illustrating the experimental procedure used to examine the autoradiographic distribution of cholinergic and etorphin binding sites following unilateral ventral root lesion (1 a) or dorsal root ligation (1 b). The sciatic nerve was ligated on both sides (2). Dorsal root ganglion (DRG), distal dorsal root (DDR), proximal dorsal root (PDR), proximal sciatic nerves (PSN), distal sciatic nerves (DSN), sensory afferent (SA) and motor efferent (ME)

nicotinic ACh binding were significantly higher on the proximal side of the ligation than on the distal side (Table 1). Muscarinic ACh binding could not be detected distally to the ligation on the control side. There was no significant change after ventral rhizotomy in the accumulation of these binding sites on the proximal side of the ligation. On the rhizotomized side, muscarinic ACh binding sites were also observed distally to the ligation, while total ACh, nicotinic ACh and QNB binding sites were unchanged on this side. No difference in accumulation of alpha-Btx binding between the proximal and distal sides of

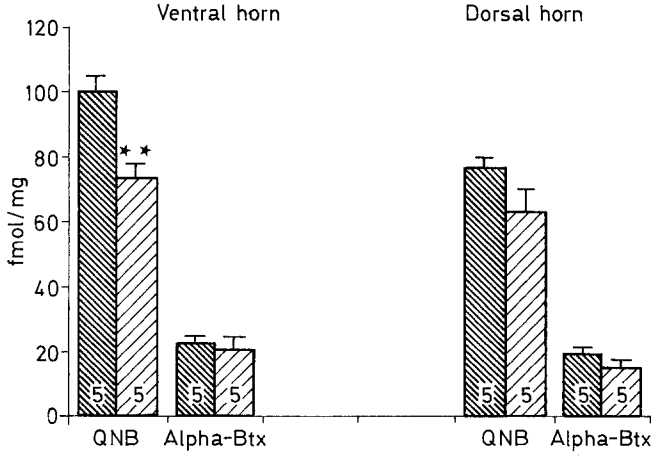

Fig. 2. Histograms of ^{3}H-quinuclidinylbenzilate (QNB) and ^{3}H-alpha-bungarotoxin (α-Btx) binding sites in cryosections of rat spinal cord after unilateral ventral root lesion. Narrow striped bars = control side, wide striped bars = ventral rhizotomized side. The figures within the bars indicate the number of rats. ** $p<0.01$

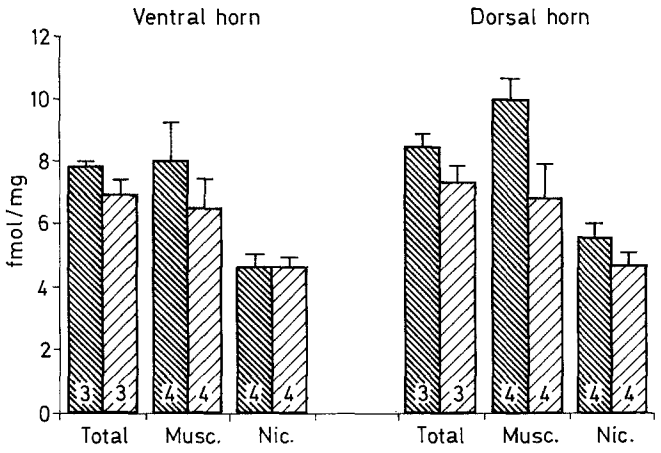

Fig. 3. Histograms of ^{3}H-acetylcholine (ACh) binding sites in cryosections of rat spinal cord after unilateral ventral root lesion. Musc = muscarinic; Nic = nicotinic. Narrow striped bars = control side, wide striped bars = ventral rhizotomized side. The figures within the bars indicate the number of rats

the ligation was observed and there was no significant change of binding sites after ventral rhizotomy (Table 1).

The accumulation of ^{3}H-etorphine binding sites was significantly higher on the proximal side of the sciatic nerve ligation than on the distal one. No significant change was observed after ventral rhizotomy (Table 1).

Table 1. Cholinergic and etorphine binding sites (fmol/mg ± SEM) proximally and distally to a sciatic nerve ligation. Control side (C), ventral rhizotomized side (VR)

Ligand	C		VR	
	Proximal	Distal	Proximal	Distal
^{3}H-QNB	40.5 ± 8.0* n = 4	18.5 ± 5.1	39.7 ± 14.2 n = 3	25 ± 12.8
^{3}H-ACh total	11.2 ± 1.7* n = 3	7.4 ± 0.4	6.4 n = 1	10.4
^{3}H-ACh muscarinic	6.4 ± 1.2* n = 3	0	9.4 ± 4.4 n = 3	3.2 ± 1.3
^{3}H-ACh nicotinic	4.6 ± 0.7* n = 4	1.6 ± 0.6	8.9 ± 3.5 n = 3	0
^{3}H-α-Btx	34.4 ± 0.1 n = 3	28.5 ± 3.4	36.8 ± 2.5 n = 3	17.2 ± 9.9
^{3}H-etorphine	31.2 ± 5.3 n = 4	9.0 ± 3.4	20.7 ± 5.0 n = 3	10.9 ± 11.2

* Signifies a statistical significant ($p \leq 0.05$) difference between the proximal and distal side

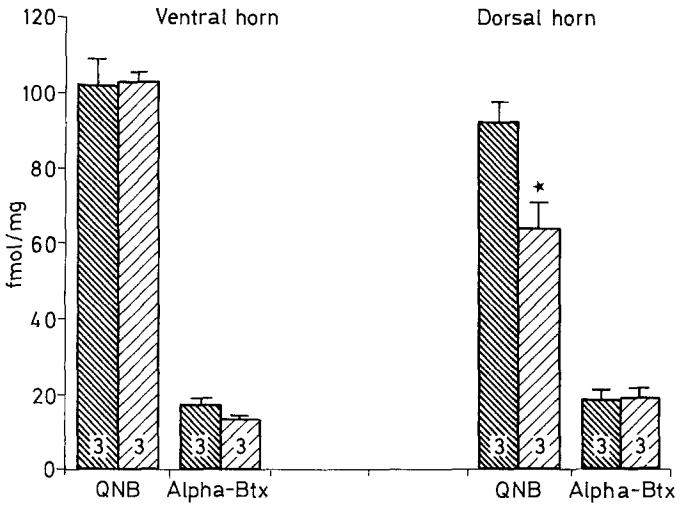

Fig. 4. Histograms of ^{3}H-quinuclidinylbenzilate (QNB) and ^{3}H-alpha-bungarotoxin (alpha-Btx) binding sites in cryosections of rat spinal cord after unilateral dorsal root ligation. Narrow striped bars = control side, wide striped bars = dorsal root ligated side. The figures within the bars indicate the number of rats. * $p < 0.05$

Dorsal root ligation

No significant differences in QNB, total ACh, muscarinic ACh, nicotinic ACh or α-Btx binding in the motor neuron area between the operated and the control sides were observed (Figs. 4 and 5).

QNB binding in the posterior horn on the operated side was significantly decreased compared to the control side (Fig. 4). There was a tendency to decreased muscarinic and nicotinic ACh binding on the operated side, but the differences were not significant (Fig. 5). No difference in alpha-Btx binding between the two sides was observed (Fig. 4).

In the dorsal root the accumulation of alpha-Btx binding sites was significantly ($p \leqslant 0.05$) higher on the distal side of the ligation than on the proximal side (proximal: 0.7 ± 0.5 fmol/mg wet weight ± SEM, distal: 3.5 ± 0.8 fmol/mg wet weight n = 4). Very similar accumulations of total ACh (3.0 fmol/mg wet weight), muscarinic ACh (2.1 fmol/mg) and nicotinic ACh (2.8 fmol/mg) were observed on both sides of the ligation. No accumulation of QNB binding sites could be detected.

The accumulation of etorphine binding sites was much more pronounced on the distal than on the proximal side of the ligation (proximal: 2.4 ± 1.2 fmol/mg wet weight, distal: 14.0 ± 3.6 fmol/mg wet weight, n = 4).

Discussion

The finding of decreased QNB binding in the motor neuron area ipsilateral to the ventral rhizotomy is in accordance with the results of a previous semi-quantitative study (Gillberg and Wiksten, 1986). This loss of receptors is prob-

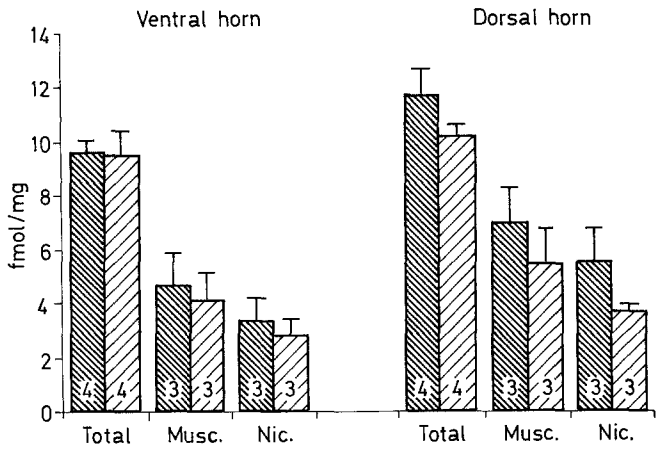

Fig. 5. Histograms of ^{3}H-acetylcholine binding sites in cryosections of rat spinal cord after unilateral dorsal root ligation. Musc = muscarinic; Nic = nicotinic. Narrow striped bars = control side, wide striped bars = dorsal root ligated side. The figures within the bars indicate the number of rats

ably a consequence of postsynaptic degeneration. The number of alpha-motor neurons is reduced after ventral rhizotomy (Kellerth, personal communication) and it is probable that muscarinic receptors are located at these neurons. The view that muscarinic ACh receptors are located at alpha-motorneurons is supported by the results of a iontophoretic study on the cat by Zieglgänsberger and Reiter (1974). The finding of a considerably higher number of binding sites for the antagonist QNB than for the agonist ACh might be due to binding to different subtypes of muscarinic receptors in the spinal cord as has been shown to be the case in rat brain (Messer and Hoss, 1987). Agonist binding sites seem to be less sensitive to axonal damage than antagonist binding sites as there was only a tendency to decreased muscarinic ACh binding in the motor neuron area after ventral rhizozomy.

The lack of reduction of nicotinic-ACh and alpha-Btx binding ipsilateral to the ventral rhizotomy renders a localization of nicotinic receptors at the α-motor neurons less likely. There is much evidence that nicotinic receptors are instead located at the Renshaw cells (Ninkovic and Hunt, 1983) which are not reduced after ventral rhizotomy (Kellerth, personal communication). As expected, there was no compensatory change in the binding pattern of any of the used ligands in the posterior horn.

The reduction of QNB binding in the posterior horn ipsilateral to the dorsal root ligation supports the concept that the QNB binding sites in this area are located presynaptically (Gillberg and Wiksten, 1986). It is plausible that cholinergic binding sites are synthesized in the dorsal root ganglion and from there transported to the posterior horn. We have, however, not been able to fully prove this idea concerning muscarinic antagonist binding as we could not detect any accumulation of QNB binding sites in the dorsal root on any side of the

ligation. That at least a subgroup of muscarinic and nicotinic binding sites in the dorsal root are transported in both antero- and retrograde directions is, however, supported by the finding that muscarinic and nicotinic ACh binding sites accumulated on both sides of the ligation. The lack of reduction of QNB binding sites in the ventral horn ipsilateral to the dorsal root ligation renders the presence of QNB binding sites located on the terminals of the primary afferents unlikely. The absence of a reduction of α-Btx binding in the posterior horn ipsilateral to the dorsal root ligation is in constrast to a non-quantitative study by Ninkovic and Hunt (1983), who found decreased binding on the operated side. Because of differences in the technical methodology it is, however, difficult to compare these two studies. A reduction of alpha-Btx binding sites in the posterior horn also would have been expected, as in the dorsal root the accumulation of binding sites was much lower proximally than distally to the ligation.

It has previously been shown by semiquantitative techniques that opioid receptors in the posterior horn are reduced after dorsal rhizotomy (Lamotte et al., 1976; Atweh et al., 1978; Ninkovic et al., 1981; Gillberg and Wiksten, 1986). The present finding of an accumulation of etorphine binding more pronounced on the proximal side of the dorsal root ligation, is further support of the concept that opioid receptors are synthesized in the dorsal root ganglion and then transported to the posterior horn.The accumulation of muscarinic, nicotinic and etorphine binding sites in the sciatic nerve both proximal and distal to the ligature suggests that these binding sites undergo both anterograde and retrograde transport. The finding of a higher accumulation of muscarinic ACh, nicotinic ACh, QNB and etorphine binding sites proximally to the ligation might be because anterograde transport is faster then retrograde or that a higher number of these binding sites are transported distally than proximally. The lack of any reduction in accumulation of binding sites in the sciatic nerve after ventral rhizotomy renders it unlikely that the binding sites are synthesized and transported in the motor neurons or elsewhere in the spinal cord and instead points to synthesis in the dorsal root ganglion. The concept that muscarinic and alpha-Btx binding sites are synthesized in the dorsal root ganglion is supported by studies by Wamsley et al. (1981) and Ninkovic and Hunt (1981), respectively. Our findings are, however, not fully in line with the results of Gulya and Kasa (1984) who found a more pronounced reduction in the accumulation of QNB binding proximally to a sciatic nerve ligature after lesion of the ventral root than after a lesion of the ganglion.

The following conclusions may be made from this study. Muscarinic antagonist binding sites are in all probability located on the perikarya of the motor neurons. There seems to be no cholinergic projection or influence from the posterior horn to the motor neuron area. Opioid receptors in the dorsal root have their origin in the dorsal root ganglion. Muscarinic antagonist binding sites in the posterior horn and in the dorsal root are probably located at the primary afferents. Cholinergic and opioid receptors in peripheral nerves are

transported in both anterograde and retrograde directions and their origin seems to be the dorsal root ganglion. Cholinergic agonist binding sites in the spinal cord seem to be less sensitive to axonal damage than antagonist binding sites.

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References

Atweh SF, Murrin LC, Kuhar MJ (1978) Presynaptic localization of opiate receptors in the vagal and accessory optic systems: an autoradiographic study. Neuropharmacology 17: 65–71

Barber RP, Phelps PE, Houser CR, Crawford GD, Salvaterra PM, Vaughn JE (1984) The morphology and distribution of neurons containing choline acetyltransferase in the adult rat spinal cord: an immunochemical study. J Comp Neurol 229: 329–346

Gillberg P-G, Wiksten B (1986) Effects of spinal cord lesions and rhizotomies on cholinergic and opiate receptor binding sites in rat spinal cord.Acta Physiol Scand 126: 575–582

Gulya K, Kasa P (1984) Transport of muscarinic cholinergic receptors in the sciatic nerve of rat. Neurochem Int 6: 123–126

Hebel R, Stromberg MW (1976) Anatomy of the laboratory rat. Williams and Wilkins, Baltimore

Laduron PM (1984) Axonal transport of receptors: coexistence with neurotransmitter and recycling. Biochem Pharmacol 33: 897–903

Laduron PM, Janssen PFM (1986) Impaired axonal transport of opiate and muscarinic receptors in streptozocin-diabetic rats. Brain Res 380: 359–362

Lamotte C, Pert CB, Snyder SH (1976) Opiate receptor binding in primate spinal cord: distribution and changes after dorsal root section. Brain Res 112: 407–412

Messer WS, Hoss W (1987) Selectivity of pirenzepine in the central nervous system. I. Direct autoradiographic comparison of the regional distribution of pirenzepine and carbamylcholine binding sites. Brain Res 407: 27–36

Ninkovic M, Hunt SP, Gleave JRW (1982) Localization of opiate and histamine H1-receptors in the primate sensory ganglia and spinal cord. Brain Res 241: 197–206

Ninkovic M, Hunt SP (1983) α-Bungarotoxin binding sites on sensory neurones and their axonal transport in sensory afferents. Brain Res 272: 57–69

Wamsley JK, Zarbin MA, Kuhar MJ (1981) Muscarinic cholinergic receptors flow in the sciatic nerve. Brain Res 217: 155–161

Zarbin MA, Wamsley JK, Kuhar MJ (1982) Axonal transport of muscarinic cholinergic receptors in rat vagus nerve: high and low affinity agonist receptors move in opposite directions and differ in nucleotide sensitivity. J Neurosci 2: 934–941

Zieglgänsberger W, Reiter C (1974) A cholinergic mechanism in the spinal cord of cats. Neuropharmacology 13: 519–527

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