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Trigeminal and Dorsal Root Ganglion Neurons Express CCK Receptor Binding Sites in the Rat, Rabbit, and Monkey: Possible Site of Opiate–CCK Analgesic Interactions

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^{125}I-Bolton–Hunter sulfated cholecystokinin-8 was used to localize and characterize cholecystokinin (CCK) receptor binding sites in trigeminal and dorsal root ganglia, and in the spinal cord of the rat, rabbit, and monkey. In the rabbit and monkey, a substantial number, 90 ± 21% and 24 ± 8%, respectively, of trigeminal and dorsal root ganglion neurons express CCK binding sites. In the spinal cord, the highest concentration of CCK receptors is found in laminae I and II, which is the major termination site of dorsal root ganglia neurons expressing CCK receptor binding sites. Neonatal capsaicin treatment of the rat results in a 70% decline in CCK receptor binding sites in laminae I and II of the spinal cord, indicating that dorsal root ganglia neurons are a major source of CCK receptors in the spinal cord. Pharmacological experiments using selective CCK-A and CCK-B receptor antagonists demonstrate that CCK-B is the prominent CCK receptor subtype in trigeminal and dorsal root ganglia neurons in the rat, rabbit, and monkey. In the rat and rabbit spinal cord, CCK-B binding sites are the prominent subtype, whereas in the monkey cord, CCK-A is the prominent receptor subtype. These results demonstrate that CCK-B receptors are expressed by a substantial percentage of dorsal root ganglion neurons at all spinal levels, and that CCK may antagonize opiate analgesia at the level of the primary afferent neuron itself.

Neurons with cell bodies located in the trigeminal ganglion or dorsal root ganglion (DRG) convey somatic sensory information from peripheral tissues to the CNS. Several neuropeptides, including cholecystokinin (CCK), have been identified within a subpopulation of these sensory neurons, suggesting that CCK may serve as a primary afferent neurotransmitter (Dalsgaard et al., 1982; Otten and Lorez, 1982; Chery-Croze et al., 1985; Faris, 1985a; Ju et al., 1987). In support of this concept, at least in some species, high levels of the peptide CCK and mRNA coding for CCK are found in DRG neurons (Cortés et al., 1990; Seroogy et al., 1990) and CCK infusion in the spinal cord produces a potent excitation of dorsal horn neurons (Jeftinija et al., 1981; Willetts et al., 1985).

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It has become increasingly evident in the last decade that the DRG neurons that convey *afferent* somatosensory information from peripheral tissues to the spinal cord are also involved in the *efferent* regulation of the peripheral tissues they innervate (C. R. Mantyh et al., 1988; P. W. Mantyh et al., 1988, 1989). Thus, the same DRG neurons that are involved in the *afferent* central transmission of sensory information are also involved in the *efferent* regulation of blood flow, gastric motility, inflammation, and the response to tissue injury (Holzer, 1988; Kruger, 1988; Mantyh et al., 1989; Barnes et al., 1990). One mechanism by which this efferent regulation occurs is the antidromic activation of sensory terminals with the resulting release of neurotransmitters from the DRG neurons. An example of this is the release of substance P from primary afferents after tissue injury in the innervated tissue, with a resulting neurogenic inflammation (Louis et al., 1989; Barnes et al., 1990; Pedersen-Bjergaard et al., 1991). What is also clear, however, is that the innervated tissue also can exert effects on the primary afferent by the release of a ligand that binds to neurotransmitter receptors expressed by the sensory nerve terminals. An example of this is the release of opiates from immunocytes that then interact with opiate receptors on sensory neurons to inhibit nociception in inflammation associated with tissue injury (Stein et al., 1990a,b).

CCK is unique among the primary afferent neurotransmitters in that it appears to function both as a neurotransmitter and as a hormone. CCK has been shown to be a neurotransmitter in the CNS and to circulate at physiologically relevant concentrations in the plasma when released from gut endocrine cells (Liddle et al., 1984, 1985). What makes this dichotomy particularly interesting is the multiple functions that have been ascribed to spinally and peripherally released CCK. Whereas CCK has been shown to modulate opiate analgesia at the spinal level (Faris et al., 1983; Watkins et al., 1984; Wiertelak et al., 1992), CCK antagonists have been shown to enhance morphine analgesia and prevent the development of tolerance to morphine analgesia (Watkins et al., 1984; Faris, 1985a; Baber et al., 1989; Wiesenfeld-Hallin et al., 1990; Kellstein et al., 1991). Because DRG neurons conduct nociceptive information from the peripheral tissues to the spinal cord, if DRG neurons do express CCK receptors, they would be uniquely situated to interact with CCK released either as a neurotransmitter in the spinal cord or as a hormone from the innervated peripheral tissue.

The studies described here were designed to identify whether trigeminal and DRG neurons express CCK receptors, to deter-

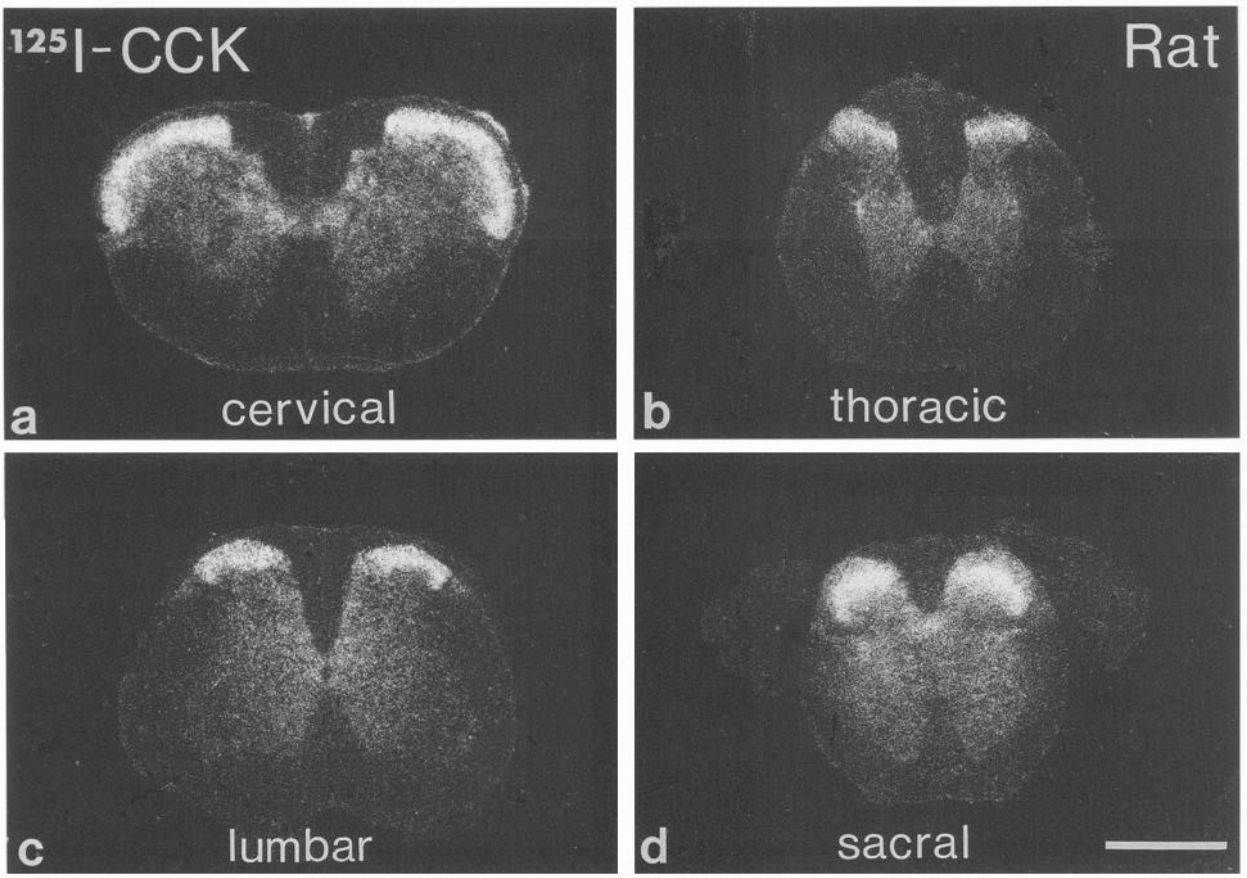

Figure 1. A series of dark-field photomicrographs illustrating the distribution of ^{125}I-CCK receptors in the rat spinal cord at the cervical (*a*), thoracic (*b*), lumbar (*c*), and sacral (*d*) spinal levels. The silver grains (*white*) correspond to regions containing a high density of ^{125}I-CCK receptors. Note that the highest density of receptors occurs in laminae I and II. Scale bar, 1.2 mm.

mine whether the expression of CCK receptors by DRG and spinal neurons is species specific, and to define the subclass of CCK receptor expressed in the trigeminal ganglion, DRG, and spinal cord.

Materials and Methods

Tissue preparation. Male Sprague–Dawley rats (250 gm; Simonsen, Gilroy, CA) were overdosed with Nembutal and perfused transcardially with 250 ml of 100 mM phosphate-buffered saline (PBS) (pH 7.4) at 4°C. Similarly, adult male New Zealand albino rabbits (Universal Animals, Inglewood, CA) were overdosed with Nembutal and perfused transcardially with 1 liter of 100 mM PBS (pH 7.4) at 4°C. Four male Macaque Nemestrina monkeys were overdosed with Nembutal and perfused transcardially with 3 liters of 100 mM PBS (pH 7.4) at 4°C. The animals were placed on ice, and the trigeminal ganglia, dorsal root ganglia, medulla, and spinal cord were rapidly removed. The tissue was blocked, frozen in Tissue-Tek, and mounted onto a brass microtome chuck. Frozen tissue sections 15 μm thick were cut on a cryostat microtome, thaw-mounted onto gelatin-coated slides, and stored at −80°C in boxes containing desiccant for up to 3 months.

Preparation of capsaicin-treated rats. The neonatal capsaicin-treated rats were prepared using a protocol identical to that previously published (Nagy et al., 1981; Mantyh and Hunt, 1985). The extent of the capsaicin lesion (50 mg/kg, given 2 d postnatally) was confirmed using a formalin wipe test. Control animals were treated in an identical manner except that they received an injection of the vehicle only. The capsaicin-treated rats and their controls were killed by overdose 6 weeks after injection.

Radioligand preparation. The radioligand used in the present study was ^{125}I-Bolton–Hunter sulfated cholecystokinin-8 purified by reversed-phase high-performance liquid chromatography to a specific activity of 2000 μCi/nmol.

Receptor binding protocol. Quantitative receptor autoradiography was performed as previously described (Mantyh and Mantyh, 1985; Niehoff, 1989). Briefly, the slide-mounted tissue sections were brought to room temperature and placed consecutively in preincubation, incubation, and wash solutions followed by a final dip in distilled water at 4°C. The preincubation and washes were performed by immersing the entire slide in the appropriate solution, whereas the incubation with the radioligand was performed by placing the slides on a flat surface and covering the sections with 1.5 ml of the incubation medium. To estimate the nonspecific binding, paired serial sections were incubated as described above except that nonradioactive sulfated CCK-8 (Bachem, Torrance, CA) was added to the incubation solution to a final concentration of 1 μM.

Cholecystokinin binding protocol. Sections were preincubated for 30 min at room temperature in 50 mM Tris-HCl (pH 7.4), 130 mM NaCl, 4.7 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, and 0.5% bovine serum albumin (BSA). The sections were then incubated in Tris saline buffer containing 0.025% bacitracin, 1 mM dithiothreitol, 2 μg/ml chymostatin, 4 μg/ml leupeptin (pH 6.5), and 100 pM ^{125}I-Bolton–Hunter CCK-8 (sulfated) for 150 min at room temperature. The sections were then washed six times for 15 min each in fresh incubation buffer containing 0.5% BSA at 4°C. The sections were then dipped twice in double-distilled H_2O at 4°C, stood upright, blown dry with cold air, and stored overnight in a slide box containing desiccant to remove any remaining moisture before exposure to autoradiographic film.

Binding protocols for other sensory neuropeptides. The binding protocols for bombesin, calcitonin gene–related peptide-α, neurokinin A, somatostatin, substance P, and vasoactive intestinal polypeptide have been published previously (Mantyh et al., 1989, 1992).

Pharmacological characterization of cholecystokinin binding sites. The CCK binding sites were characterized by performing the binding as described above except that increasing amounts of either the CCK-A receptor antagonist L-364,718 (also known as MK-329 and devazipide) or the CCK-B receptor antagonist L-365,260 (Merck, Sharp and Dohme) were added to the incubation medium (Hill et al., 1987, 1988). The slides were then processed for autoradiography as described above and

Table 1. Relative density of CCK binding sites

Level	Rat	Rabbit	Monkey
Trigeminal			
ganglion	28 ± 4	33 ± 5	ND
Brain Stem			
Sol	ND	87 ± 9	100 ± 4
Cervical			
DRG	38 ± 4	46 ± 5	62 ± 5
I	86 ± 7	59 ± 5	41 ± 4
II	86 ± 7	100 ± 3	45 ± 3
III–IV	24 ± 6	47 ± 3	11 ± 2
V–VI	21 ± 3	14 ± 1	2 ± 1
VII–IX	9 ± 6	11 ± 3	1 ± 1
X	23 ± 4	14 ± 2	8 ± 1
Thoracic			
DRG	32 ± 6	43 ± 5	60 ± 3
I	85 ± 6	69 ± 6	40 ± 3
II	85 ± 6	97 ± 5	58 ± 4
III–IV	23 ± 3	41 ± 4	14 ± 2
V–VI	19 ± 4	17 ± 4	13 ± 1
VII–IX	17 ± 4	20 ± 2	7 ± 2
X	17 ± 4	21 ± 2	12 ± 1
IL	19 ± 4	36 ± 3	13 ± 2
Lumbar			
DRG	40 ± 4	44 ± 5	67 ± 7
I	100 ± 6	46 ± 3	45 ± 4
II	100 ± 6	79 ± 4	60 ± 3
III–IV	18 ± 4	39 ± 4	12 ± 2
V–VI	18 ± 3	15 ± 3	9 ± 1
VII–IX	20 ± 4	19 ± 2	5 ± 2
X	22 ± 3	19 ± 3	8 ± 1
Sacral			
DRG	31 ± 5	38 ± 3	58 ± 5
I	74 ± 5	56 ± 4	29 ± 3
II	74 ± 5	90 ± 3	55 ± 4
III–IV	27 ± 3	45 ± 4	14 ± 2
V–VI	24 ± 3	18 ± 2	8 ± 1
VII–IX	9 ± 3	23 ± 2	7 ± 1
X	16 ± 3	21 ± 2	8 ± 1
Onuf's	9 ± 3	23 ± 2	7 ± 1

Data are relative density of CCK binding sites in the trigeminal ganglia, DRG, caudal aspect of the nucleus of the solitary tract, and the spinal cord of the rat, rabbit, and macaque monkey. Densitometric readings from each region were taken from at least three different animals, averaged, and corrected for the nonlinearity of the film as previously described using autoradiographic standards (Mantyh et al., 1988). Values are expressed as a percentage of the highest density of binding found for each species, that is, rat, laminae I and II of the lumbar spinal cord; rabbit, laminae II or the cervical spinal cord; monkey, caudal aspect of the nucleus solitarius. ND, not determined; Sol, caudal aspect of the nucleus of the solitary tract; IL, intermediolateral group; Onuf's, Onuf's nucleus.

the density of silver grains was analyzed with the IMAGE (version 1.37) image analysis program to quantify the percentage inhibition of saturable ^{125}I-CCK binding at each concentration of CCK antagonist.

Autoradiography. The tissue sections were processed for autoradiography by placing them in apposition to LKB tritium-sensitive film with iodinated standards (Amersham). After 1–3 weeks, the LKB film was developed in D-19 developer, fixed, and washed. In selected sections where a higher degree of histological resolution was desired, the sections were fixed using paraformaldehyde vapors and then processed for standard emulsion-dipped autoradiography. Finally, the emulsion-dipped autoradiograms were developed, placed in Carnoy's fixative for 3 hr, Nissl-stained, and mounted with Histoclad. Dark-field and bright-field photomicrographs were then taken of the emulsion-dipped and counterstained sections, respectively. This approach allowed us to generate three complementary images: the LKB autoradiograms were analyzed by quantitative densitometry, the emulsion-dipped slides provided detailed histological resolution of the binding sites, and the counterstained sections allowed identification of the area and in some cases the cell type expressing the specific binding sites. Controls for chemographic artifacts were made by performing the binding exactly as described except that the radioligand was omitted from the incubation medium.

Densitometry. In order to quantify the density of ^{125}I-CCK binding sites in the capsaicin experiments and in the spinal cord, microdensitometry with tritium-sensitive film was performed as previously described (Mantyh et al., 1988). For the pharmacological characterization of the receptors, the Macintosh-based IMAGE (version 1.37) software program was used. $P < 0.05$ was considered significant.

Statistical analysis. ^{125}I-CCK receptor density in normal and capsaicin-treated rats was analyzed using the Students t test for independent samples to determine statistical significance. Seven measurements of the spinal cord binding densities in normal and capsaicin-treated rats were used to determine the average value for each lamina. The averages were then used to compute the statistical significance between the normal and capsaicin-treated tissues.

Results

CCK binding sites in the normal and capsaicin-treated rat

In the normal rat, the highest density of specific CCK binding sites was present in laminae I and II of the spinal cord at the cervical, thoracic, lumbar, and sacral levels (Fig. 1). The density of binding sites in laminae III–X was lower, although detectable levels of specific binding could be detected in all laminae. The cells of origin of at least a substantial portion of these CCK receptor binding sites appear to be the primary afferents themselves because DRGs contain a moderate density of receptor binding sites (Table 1), and prenatal capsaicin treatment significantly reduced the density of CCK receptor binding sites in several laminae (Fig. 2). It should be noted that it was not possible to estimate the percentage of DRG neurons expressing CCK binding sites in the rat because of the low density of CCK binding sites and the subsequent inability to obtain the single-cell resolution of the binding sites needed to estimate the percentage of DRG neurons expressing CCK receptors. Comparisons of the inhibition of CCK binding in laminae I and II of the spinal cord using CCK-A– and CCK-B–selective antagonists suggested that the majority of binding sites present in these laminae, which capsaicin experiments demonstrate arise from DRG neurons, are of the CCK-B subtype (Fig. 3*a*).

CCK binding sites in the rabbit DRGs and spinal cord

A high density of ^{125}I-CCK binding sites was found in the trigeminal ganglion (Fig. 4) and the cervical (Fig. 5*a–c*), thoracic (Fig. 5*d–f*), lumbar, and sacral DRG. Approximately 90 ± 21% of the trigeminal ganglion neurons and DRG neurons at all spinal levels expressed saturable ^{125}I-CCK binding sites. The neurons that did not bind ^{125}I-CCK were predominantly large to medium in size and were distinct as they were stained much darker than other neurons in the ganglion. It should be noted that the majority of the larger neurons that did bind ^{125}I-CCK had a significantly lower density of binding than that found over the smaller and medium-sized neurons that displayed ^{125}I-CCK binding sites.

The pattern of CCK binding sites observed in the spinal cord and DRGs of the rabbit was similar to that seen in the rat. In the spinal cord the highest levels of ^{125}I-CCK binding were found in laminae I and II (Fig. 6, Table 1). The rabbit brainstem was

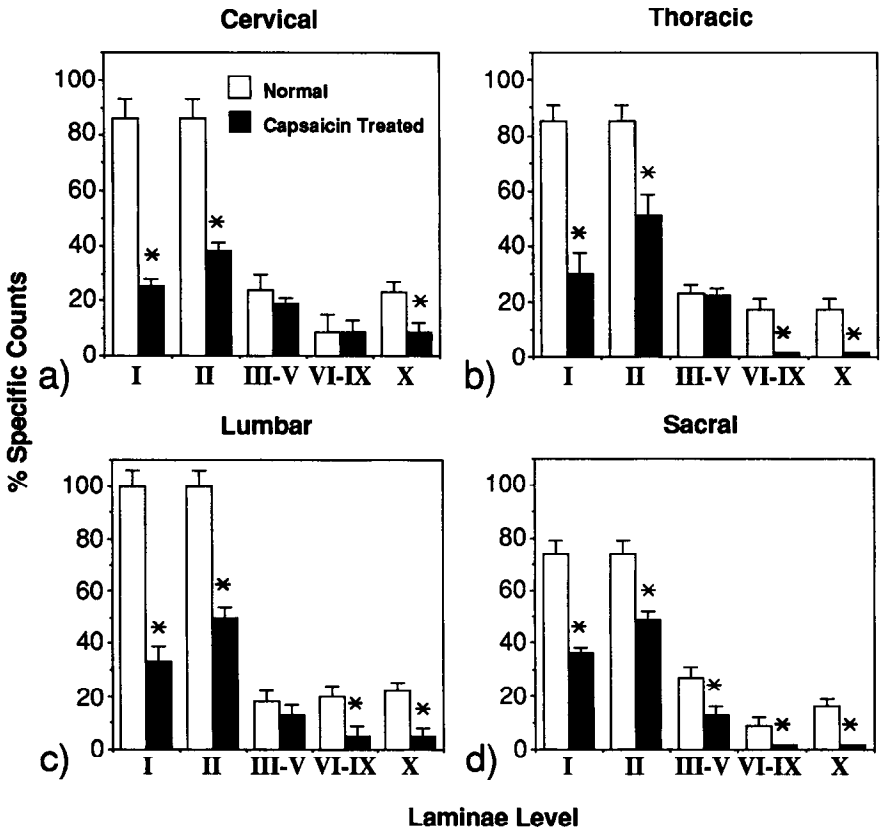

Figure 2. Comparison of the optical density of ^{125}I-CCK binding sites in the spinal cords of five normal and five neonatally capsaicin-treated rats. *a–d* are from the cervical, thoracic, lumbar, and sacral spinal regions, respectively. The optical density of silver grains produced by bound ^{125}I-CCK is expressed as a percentage of the density found over laminae I and II in the lumbar region of the spinal cord, defined as 100%, and the other densities are expressed as a percentage of this maximum. Note that at each spinal level the most significant difference in concentration of ^{125}I-CCK bound occurs in laminae I and II. Less dramatic but significant differences between the normal and capsaicin-treated animals were also found in laminae VI–X at the cervical, thoracic, lumbar, and sacral levels. The *asterisk* indicates statistical significance, $0.05 > p > 0.001$, compared to control animals.

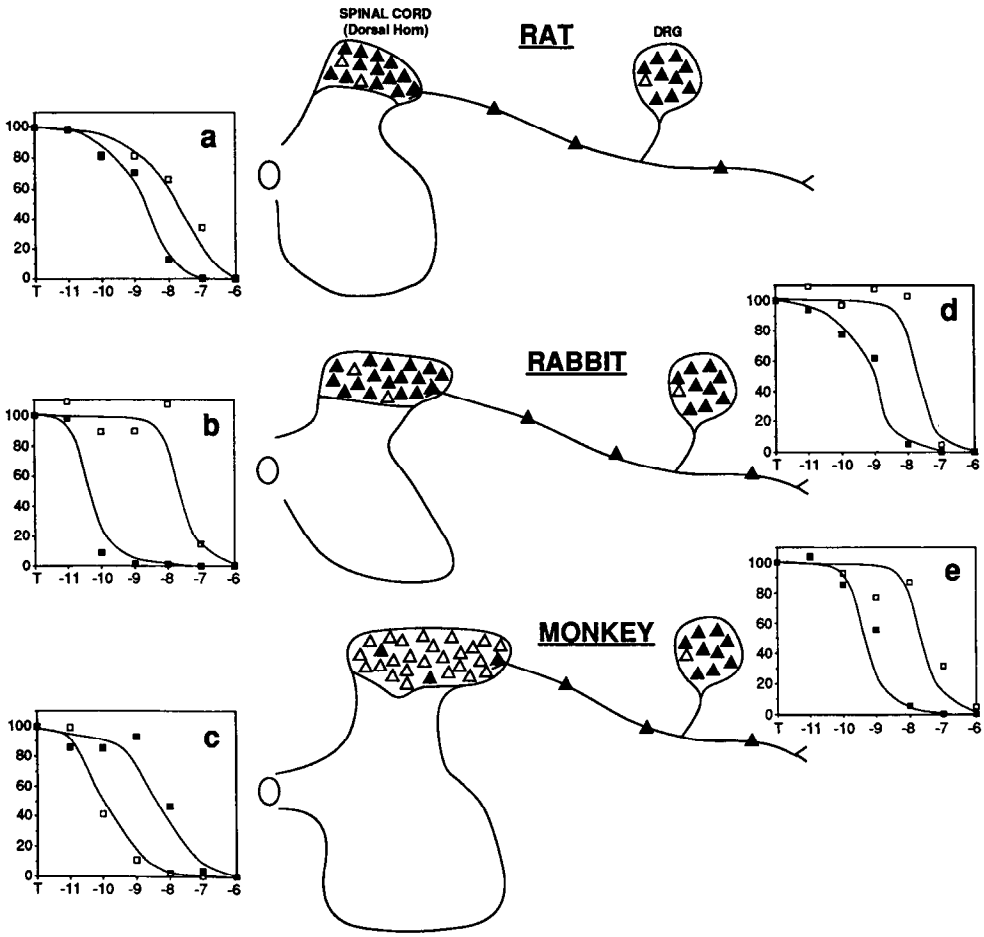

Figure 3. Schematic diagram showing the distribution of CCK-A (△) and CCK-B (▲) receptors in the rat, rabbit, and monkey spinal cord and DRG. Next to each spinal cord or DRG is a displacement curve that was used to define the subtype of CCK receptors expressed by rat spinal cord (*a*), rabbit spinal cord (*b*), monkey spinal cord (*c*), rabbit DRG (*d*), and monkey DRG (*e*). The displacement curves were generated by incubating serial sections of either lumbar cord or lumbar DRG in 100 pM ^{125}I-CCK with varying concentrations of either a CCK-A receptor antagonist (L-364,718; □) or a CCK-B receptor antagonist (L-365,260; ■) and then densitometrically analyzing the resulting autoradiograms. Each point in the displacement curves is an average of at least three experiments where the SEs were less than 15% of the mean for all points. A displacement curve is not included for the rat DRG, for although capsaicin experiments suggest these DRG neurons also synthesize and transport CCK receptors to the spinal cord, because of the low density of CCK binding sites in the rat DRG, it was not possible to generate a displacement curve for this tissue.

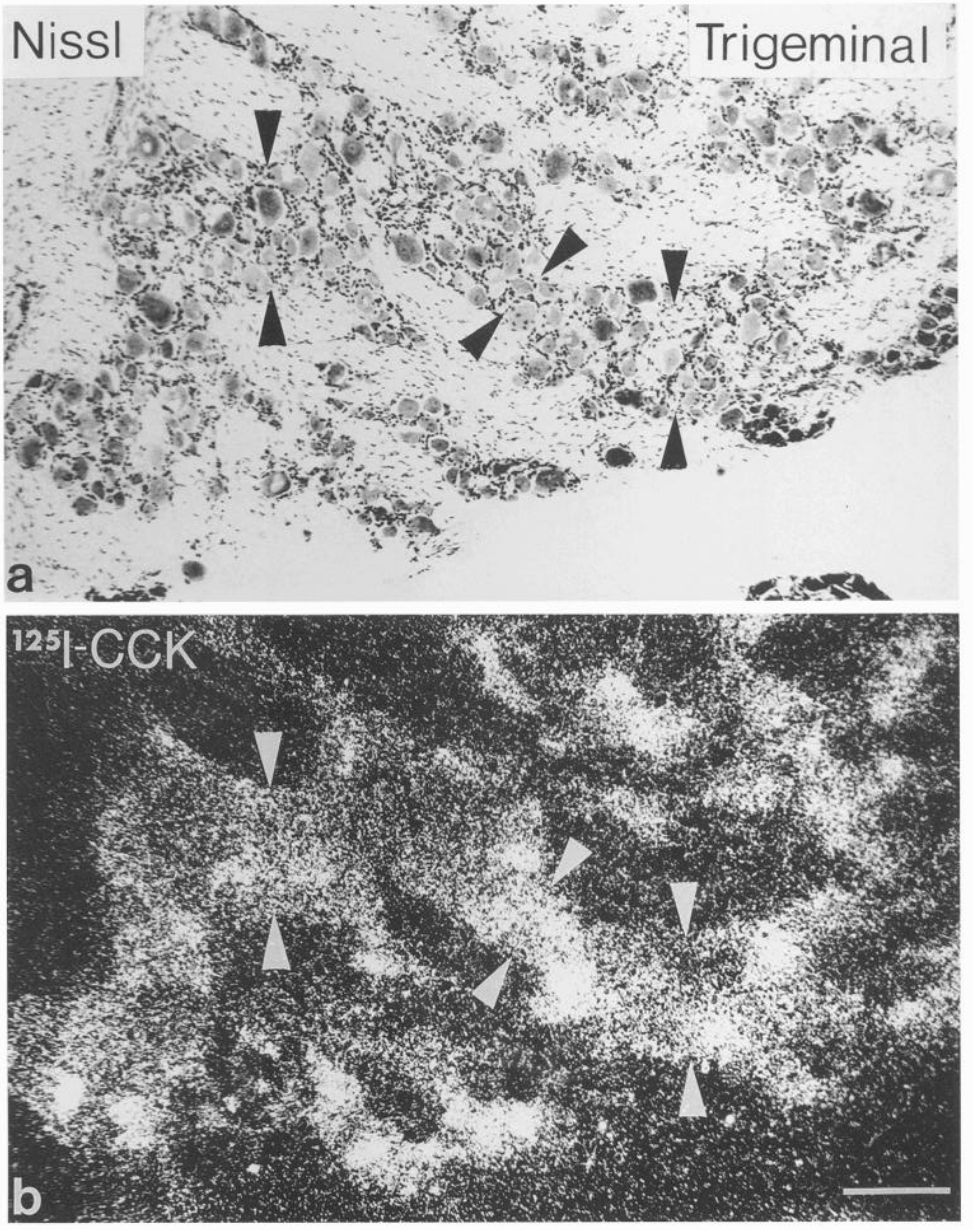

Figure 4. Localization of ^{125}I-CCK receptor binding sites in the rabbit trigeminal ganglion. *a*, Bright-field photomicrograph of a rabbit trigeminal ganglion section Nissl stained to visualize the neurons within the ganglion. *b*, Dark-field photomicrograph of the same trigeminal ganglion section dipped in nuclear emulsion, after incubation with the ^{125}I-CCK ligand, in order to show the ^{125}I-CCK binding sites. The *arrowheads* in the bright- and dark-field photomicrographs point to the same groups of neurons. Note that the silver grains are localized primarily over the neurons and not over the intercellular spaces or glia cells. Scale bar, 0.1 mm.

also processed for receptor autoradiography and expressed high levels of ^{125}I-CCK binding in the caudal aspect of the nucleus of the solitary tract (Fig. 6*a*). Lower levels of ^{125}I-CCK binding were seen throughout the rest of the spinal cord in laminae III–X. In general, the binding in these areas was approximately 10–30% of that found in either lamina I or II.

Comparisons of the inhibition of ^{125}I-CCK binding by CCK-A and CCK-B receptor antagonists suggest that the CCK-B receptor subtype is the prominent subtype present in laminae I and II of the spinal cord and in the DRG of the rabbit (Fig. 3*b,d*). It should be noted, however, that even at concentrations of the CCK-B antagonist L-365,260 at which the majority of DRG neurons show no binding, a small (10%) but significant number of DRG neurons still exhibit saturable CCK binding, suggesting that a small percentage of these neurons may express the CCK-A subtype of receptor.

CCK binding sites in the monkey DRGs and spinal cord

The monkey DRGs contained a high to moderate density of ^{125}I-CCK binding sites. Approximately 24 ± 8% of all the trigeminal ganglia neurons and DRG neurons expressed ^{125}I-CCK binding sites. As was the case in the rabbit, the majority of DRG neurons expressing CCK binding sites were small to medium in size (Fig. 7).

In the monkey, as in the rat and rabbit, high concentrations of binding sites were found in laminae I and II of the spinal cord (Fig. 8, Table 1). Higher levels are seen in lamina II than in lamina I, with the highest amount found in the caudal aspect of the nucleus of the solitary tract.

Analysis of the inhibition of CCK binding by CCK-A and CCK-B receptor antagonists reveals that the CCK-A subtype of receptor is prominent in the monkey spinal cord neurons and

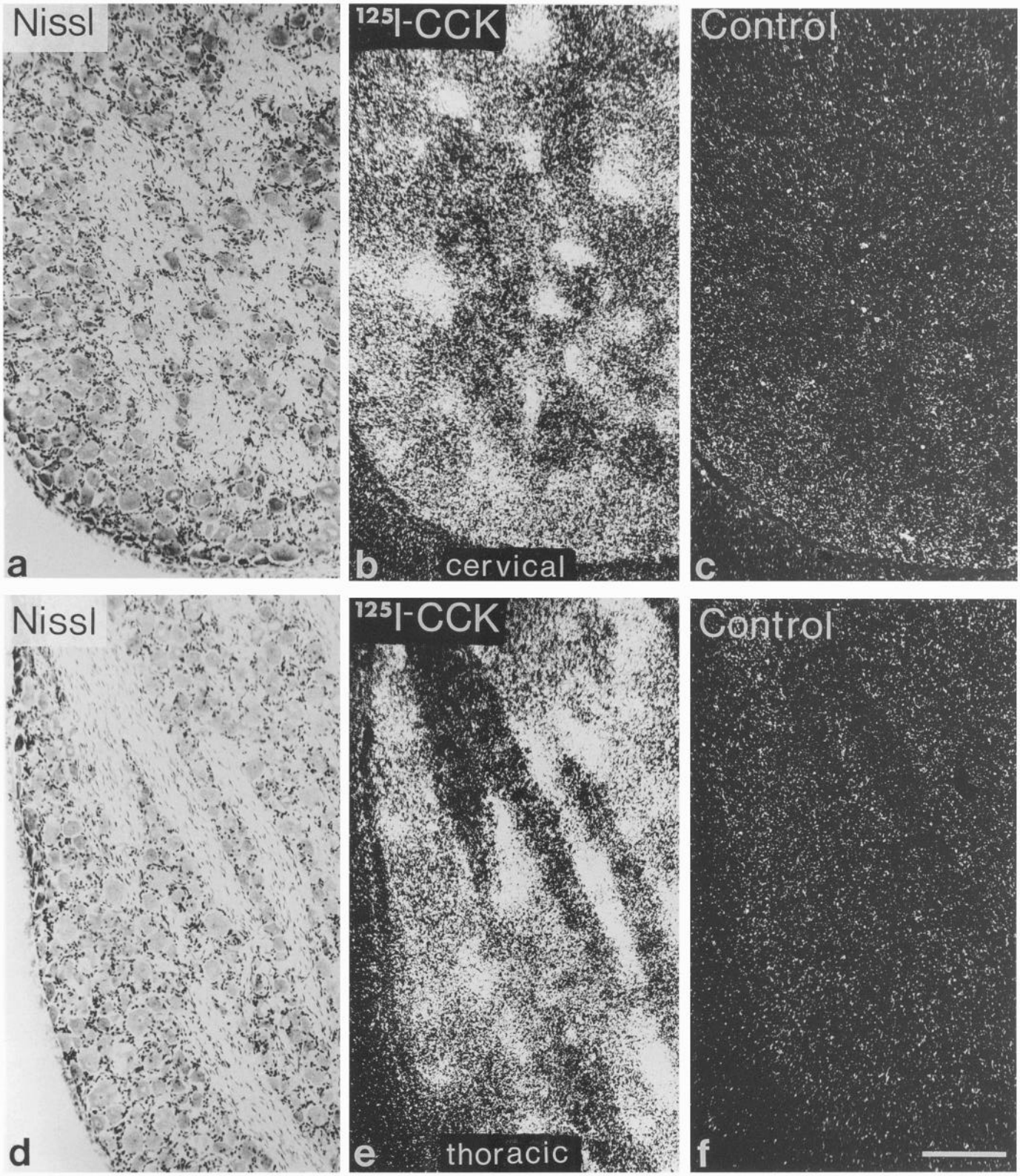

Figure 5. The distribution of ^{125}I-CCK binding sites within the rabbit cervical and thoracic DRG. *a* and *d*, Bright-field photomicrographs of Nissl-stained cervical (*a*) and thoracic (*d*) ganglia, respectively, showing the distribution of neurons within the ganglion. *b* and *e*, Dark-field photomicrographs of the same cervical (*b*) and thoracic (*e*) ganglia dipped in nuclear emulsion after incubation with ^{125}I-CCK. Note that the silver grains are localized only to regions containing neurons, with relatively little binding occurring over regions that do not contain neurons. *c* and *f*, Serial sections of the cervical and thoracic ganglia that have been treated identically to those seen in *b* and *e* except that nonradioactive sulfated CCK-8 was added to the incubation medium to a final concentration of 1 μM. In order to obtain the specific binding, the binding found in *c* and *f* was subtracted from the binding observed in *b* and *e*, respectively. Scale bar, 0.15 mm.

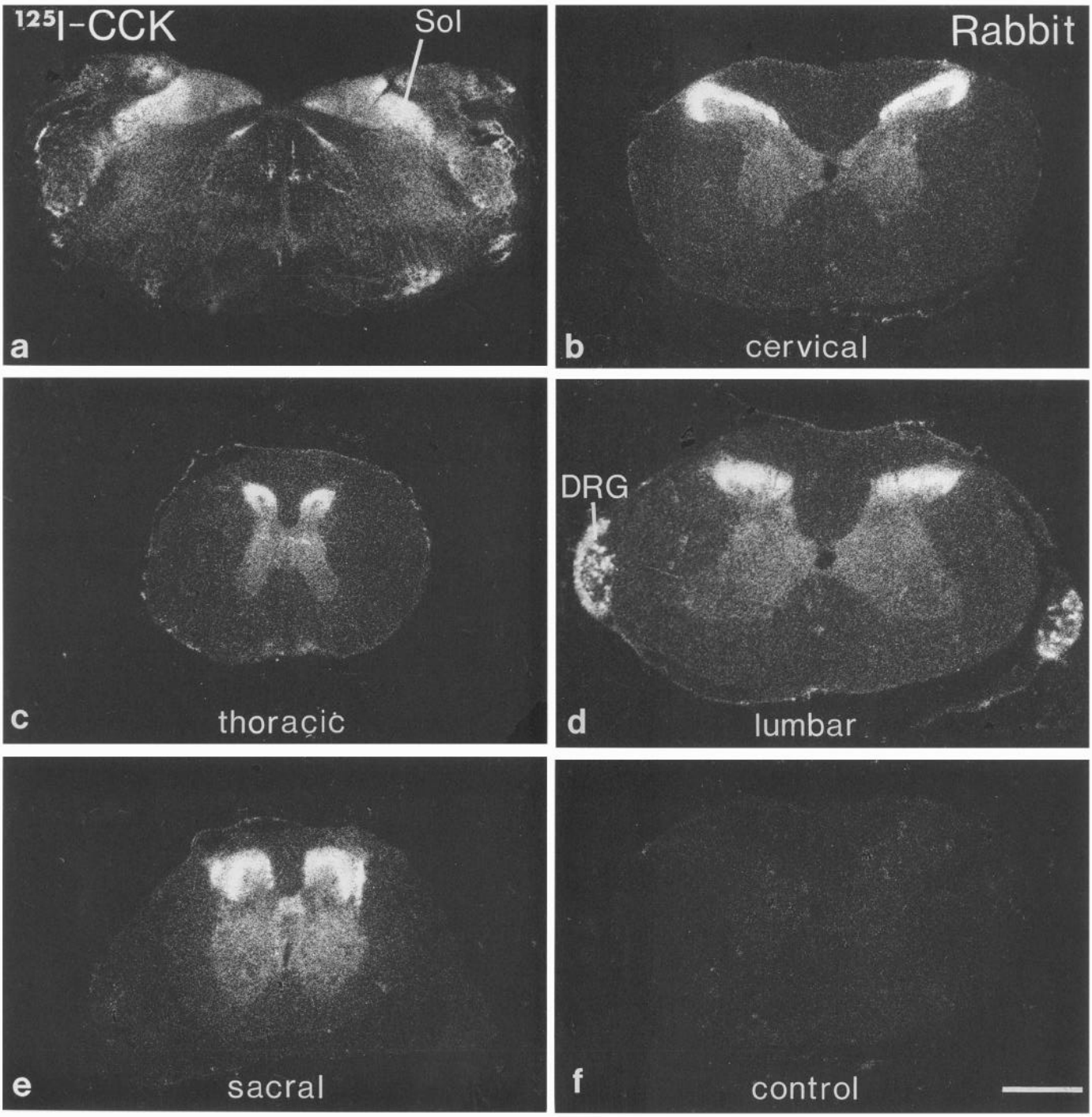

Figure 6. Autoradiographic localization of ^{125}I-CCK sites in rabbit brainstem and spinal cord. Dark-field photomicrographs were made from tritium-sensitive film that was apposed to rabbit brainstem (*a*) and sections of the cervical (*b*), thoracic (*c*), lumbar (*d*), and sacral (*e*) spinal cord. Note that in the brainstem (*a*) the region with the highest density of binding sites occurs in the medial aspect of the nucleus of the solitary tract. In the spinal cord, the highest density of binding is found over laminae I and II. Note also the binding associated with DRG neurons in *d*. The control section (*f*) was a section adjacent to *d* except that nonradioactive sulfated CCK-8 (1 μM) was present in the incubation medium. Scale bar, 1.3 mm.

that the prominent subtype in the DRG neurons is the CCK-B receptor (Fig. 3*c,e*). Similar to the results obtained using rabbit tissue noted above, a small (10%) but significant number of monkey DRG neurons still exhibit binding sites even at concentrations of CCK-B at which the majority of DRG neurons show no binding, suggesting that a small percentage of these neurons may express the CCK-A subtype of receptor (Fig. 9).

Binding sites for other sensory neuropeptides in DRG neurons

Receptor binding sites for bombesin, calcitonin gene–related peptide-α, neurokinin A, somatostatin, substance P, or vasoactive intestinal polypeptide were not detected in the DRG of the rat, rabbit, or monkey (Table 2). However, for all of these same neuropeptides, high levels of specific binding were ob-

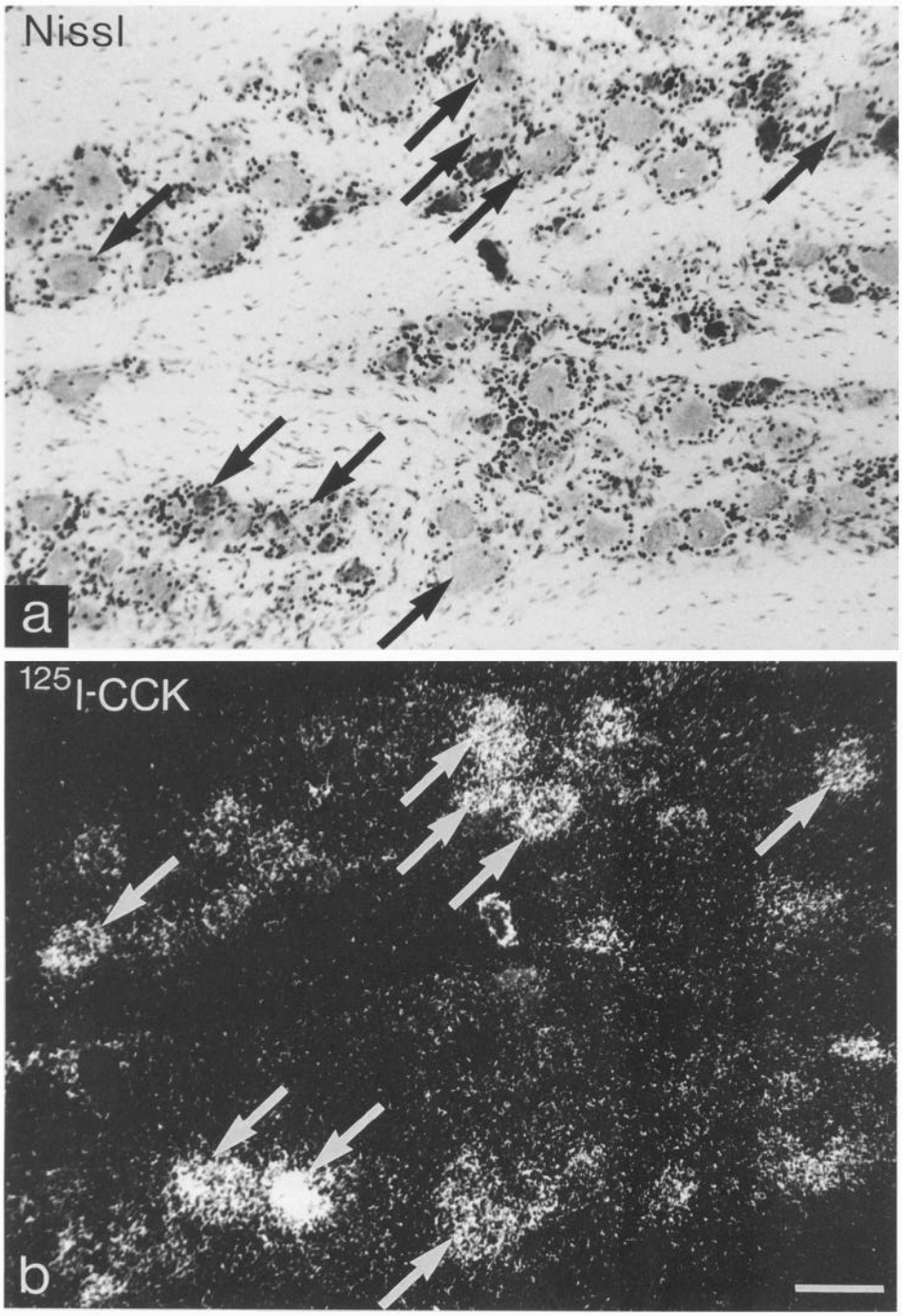

Figure 7. Distribution of ^{125}I-CCK binding sites over neurons located within the monkey trigeminal ganglion. *a*, High-power bright-field photomicrograph of a Nissl-stained section. *b*, Dark-field photomicrograph of the same section as in *a*, with *arrows* indicating neurons that exhibit both ^{125}I-CCK binding sites (*b*) and Nissl stain (*a*). Scale bar, 0.1 mm.

served in the rat, rabbit, and monkey spinal cord. These data suggest that if receptor binding sites for these neuropeptides are present on DRG neurons, either they are expressed at a level below that which can be detected using the current method or they are a different subclass of receptor that can only be detected using a different set of binding conditions or a different radioligand.

Discussion

CCK receptors expressed by DRG neurons

This report has demonstrated that a significant percentage of trigeminal and DRG neurons express CCK receptor binding sites and terminate in the spinal cord. Previous reports have noted that there are striking differences in the subclass of CCK receptor expressed in rat versus human or monkey spinal cord. Whereas CCK-B is the predominant subclass in the rat spinal cord; in the monkey and human, CCK-A is the predominant subclass (Hill et al., 1988). In contrast, the present report shows that in the rat, rabbit, and monkey, CCK-B is the predominant form of CCK receptor in DRG neurons. It is somewhat paradoxical that there is a species difference in the spinal cord, since DRG neurons, which are a major source of CCK receptors in the spinal cord, express CCK-B receptors in all species examined (Fig. 3). This apparent paradox has been resolved by the ob-

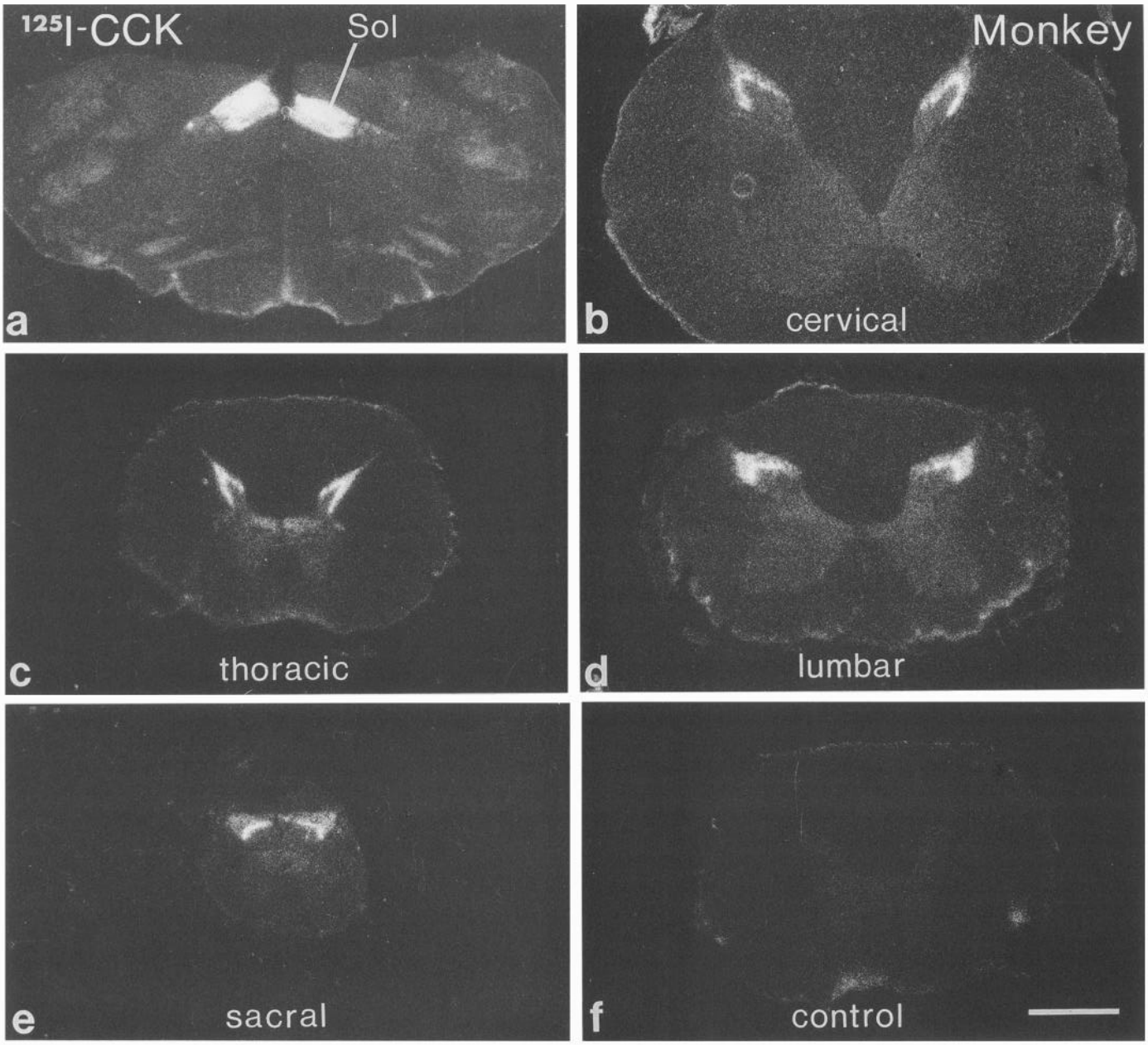

Figure 8. ^{125}I-CCK binding sites in the monkey brainstem and spinal cord. *a–e*, Dark-field photomicrographs of ^{125}I-CCK binding to the caudal aspect of the nucleus of the solitary tract (*Sol*) and laminae I and II in the monkey brainstem (*a*), and cervical (*b*), thoracic (*c*), lumbar (*d*), and sacral (*e*) spinal cord, respectively. *f*, Control section where nonradioactive sulfated CCK-8 was added to the incubation medium to a final concentration of 1 μM. Scale bar, 1.2 mm.

servation that even in the primate spinal cord, where CCK-A receptors are the predominant subclass, there is still a substantial number of CCK-B receptors in laminae I and II of the spinal cord, where the DRG neurons expressing CCK-B receptors are known to terminate. Thus, the dramatic species differences appear to be due to an increase in the expression of CCK-A receptors by neurons intrinsic to the spinal cord in monkey and man and a concomitant decrease in the percentage of DRG neurons that express CCK receptors (90% in rabbit vs 24% in monkey) rather than a change in the subclass of CCK receptor expressed by DRG neurons; that is, DRG neurons in the rat, rabbit, and monkey all express CCK-B receptors.

Neurons located in the trigeminal ganglion and the DRG are unique in that the central axon terminates in the brainstem or spinal cord within the CNS, whereas the peripherally directed axon, which innervates peripheral tissues such as skin, stomach, and muscle, is entirely outside the CNS. Previous data on another class of G-protein–coupled receptors expressed by trigeminal and DRG neurons, the opiate receptors, have shown that these receptors are transported both centrally within the axon to the spinal cord and also peripherally to terminals in the innervated tissue (Zarbin et al., 1990). It has also recently been shown that opiate receptors present in the peripheral terminals of DRG neurons are functional in that when occupied by an opiate agonist they produce a potent analgesia (Stein et al., 1990a,b; 1991). Because the present data demonstrate that CCK

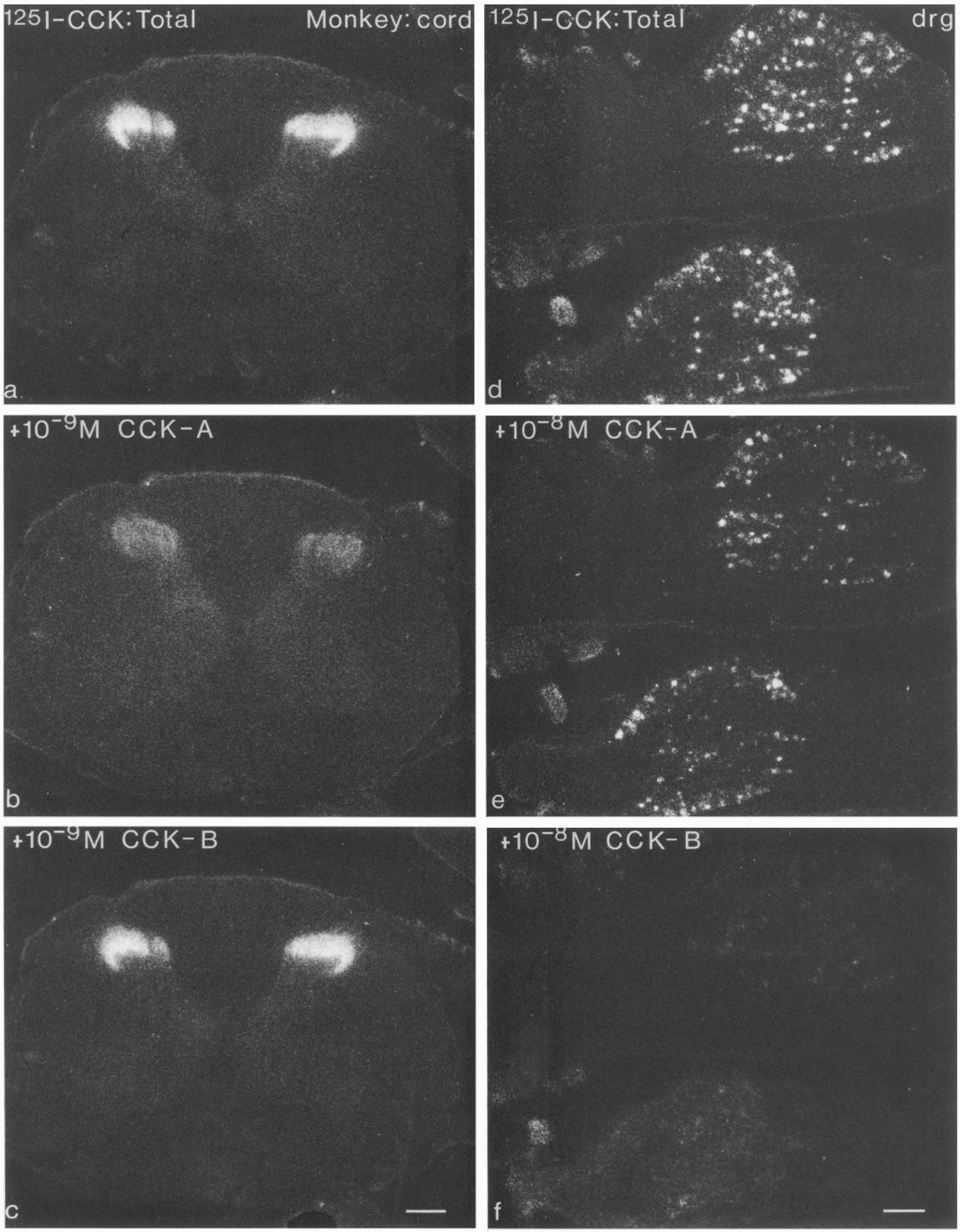

Figure 9. Autoradiographic localization of CCK receptors in serial sections of either monkey spinal cord (*a–c*) or monkey DRG (*d–f*) in the presence of a CCK-A antagonist (L-364,718) (*b* and *e*) or a CCK-B antagonist (L-365,260) (*c* and *f*). Scale bars: *a–c*, 0.9 mm; *d–f*, 1.0 mm.

Table 2. Receptors for sensory neuropeptides expressed by DRG neurons

Neuropeptide	Rat	Rabbit	Monkey
Bombesin	–	–	ND
CGRP-α	–	–	–
CCK	[+]	+++++	++
Neurokinin A	–	–	–
Somatostatin	–	–	ND
Substance P	–	–	–
VIP	–	–	–

Location and density of receptor binding sites for several sensory neuropeptides on the dorsal root ganglia neurons in the rat, rabbit, and monkey. Values are expressed as the percentage of DRG neurons that express detectable binding sites for each neuropeptide where – is undetectable; +, 1–20%; ++, 21–40%; +++, 41–60%; ++++, 61–80%; +++++, 81–100%; and ND, not determined. [+] indicates that while capsaicin experiments suggest that these DRG neurons also synthesize and transport CCK receptors to the spinal cord, because of the low density of CCK receptors it was not possible to obtain a reasonable estimate of the percentage of DRG neurons expressing CCK receptors. CGRP-α, calcitonin gene–related peptide-α; VIP, vasoactive intestinal polypeptide.

receptors are also synthesized by DRG neurons, these CCK receptors are probably also transported both centrally and peripherally, and thus can be expected to be found at both the central and peripheral terminals of DRG axons. These data suggest that both opiate and CCK-B receptors may be found in the same DRG nerve terminal in either the spinal cord or peripheral tissues, and thus CCK modulation of opiate analgesia may occur with either centrally or peripherally administered opiates.

Sources of CCK that might occupy DRG CCK receptors

There are at least three possible sources of CCK that occupy CCK receptors expressed by DRG neurons. The first possible source is the DRG neurons themselves. It has been shown that CCK immunoreactivity is found in approximately 10% of DRG neurons in nearly every mammalian species examined including the rat, guinea pig, rabbit, cat, monkey, and man (Otten and Lorez, 1982; Hökfelt et al., 1988). One inexplicable finding, however, is that whereas CCK-like immunoreactivity can be detected by immunohistochemistry in the rat, CCK-like immunoreactivity could not be detected using radioimmunoassay in the same experiments using the same antibodies at significantly lower concentrations (Marley et al., 1982; Schultzberg et al., 1982). These data suggested that, at least in the rat, the CCK immunoreactivity in the DRG might not be authentic CCK but rather another peptide, such as calcitonin gene–related peptide, with which the antibodies raised against CCK were cross-reacting (Hökfelt et al., 1988; Ju et al., 1986, 1987). Indeed, this appears to be the case, as it has been shown that whereas a substantial population of guinea pig DRG neurons contain high levels of mRNA coding for CCK, CCK mRNA is not detectable in the rat DRG (Cortës et al., 1990; Seroogy et al., 1990). Release of CCK from DRG neurons might then modulate the further release of CCK and other neurotransmitters from primary afferents. As noted in Table 2, autoreceptors for sensory neuropeptides on DRG neurons do not appear to be a general (or at least detectable) feature of DRG neurons. Why CCK might be unique in this respect is not readily apparent, but it may provide, as is discussed below, an intrinsic mechanism to regulate the analgesic actions of opiates, which are known to affect the discharge patterns of DRG neurons profoundly (Stein et al., 1988, 1990a,b).

Other sources of CCK that might interact with CCK receptors expressed by DRG neurons are neurons such as midbrain periaqueductal gray neurons (Skirboll et al., 1982) and raphe magnus neurons (Mantyh and Hunt, 1984). These neurons have been shown to contain CCK-like immunoreactivity and project to the spinal cord. CCK released from these populations of neurons could exert a presynaptic effect on DRG nerve terminals that enter the spinal cord and express CCK receptor binding sites. Presynaptic inhibition of incoming sensory input by opiates has previously been demonstrated (Levine and Taiwo, 1989), and in light of the present observation that these same nerve terminals also contain CCK receptors, a similar direct modulation of incoming primary afferents by CCK is likely.

A third source of CCK that may occupy the CCK receptors expressed by DRG neurons is CCK released in peripheral tissues. CCK is both a hormone and a neurotransmitter. The major source of circulating CCK is the small intestine, and plasma concentrations of CCK have been reported to be approximately 5–10 pM after a meal (Liddle et al., 1984, 1985).

CCK involvement in regulating opiate actions

Previous studies have demonstrated that CCK and opiates have antagonistic actions on nociceptive stimuli (Faris et al., 1983; Watkins, 1984; Faris, 1985b; Hong and Takemori, 1989; Lee et al., 1990). CCK agonists appear to antagonize opiate-induced analgesia, whereas CCK-B antagonists appear to potentiate the effects of morphine and can even prevent the development of morphine tolerance (Dourish et al., 1990; O'Neill et al., 1990; Wiertelak et al., 1992). Interestingly, CCK agonists or antagonists do not appear to affect baseline levels of pain responsivity, but rather only show an effect in the presence of opiates (Baber et al., 1989). While the mechanism by which CCK and opiates interact is not clear, several aspects of this interaction are known.

The most obvious explanation, that CCK inhibits the binding of opiates to their receptors or that opiates inhibit the binding of CCK to CCK receptors, has received little experimental support (Baber et al., 1989; Hughes et al., 1990), with the exception of a recent report (Slaninova et al., 1991). Instead, opiates and CCK appear to be acting on a common neural pathway, such as the DRG neuron. Previous reports have demonstrated that DRG neurons express several subtypes of opiate receptors (Stein et al., 1988, 1990b), and when opiates bind to these opiate receptors, a potent analgesia results (Stein et al., 1990b, 1991). The present study has demonstrated that CCK receptors are also present on DRG neurons, suggesting that in addition to spinal and supraspinal sites, opiate–CCK interactions can take place directly on the incoming spinal afferent axon. What is less clear is the intracellular mechanism by which CCK might modulate opiate analgesia on DRG neurons. Opioids that selectively activate μ-opiate receptors, the receptors that mediate the classic analgesic action of morphine, activate an inhibitory G-protein that in turn inhibits adenylate cyclase and decreases the production of cAMP (Taiwo et al., 1992). This raises the threshold of the terminal of the nociceptor, causing the firing rate to decrease and a subsequent decrease in pain. Whereas the second messenger response induced by CCK-B receptors has not been completely elucidated, both the cAMP and calcium second messenger systems can indirectly regulate the phosphorylation of a cAMP response element binding protein (Dash et al., 1991; Sheng et al., 1991; Singh et al., 1991), suggesting that the interaction of the opiates and CCK in regulating a single nociceptor's function may ultimately be related to opposing intracellular events in the same DRG neuron. This in turn may alter

the threshold of the nociceptor, which will directly influence the frequency and amount of transmitters that signal nociceptive events in the spinal cord.

CCK involvement in other functions

Recent data have demonstrated that CCK-B antagonists administered either orally or subcutaneously are potent anxiolytic compounds in mice, rats, and primates (Hughes et al., 1990; Singh et al., 1991). While the majority of CCK-B receptors are present in the brain, CCK-B receptors are present on DRG neurons and would be expected to be occupied by a CCK-B antagonist administered either orally or subcutaneously. Since a CCK-B antagonist would be expected to potentiate any endogenous opiate-induced analgesia on DRG neurons, the present data suggest a second site (other than the CNS where the great majority of CCK-B receptors are located) at which CCK-B antagonists may exert their anxiolytic activities.

Summary

This report has demonstrated that a substantial population of trigeminal ganglion neurons and DRG neurons express primarily CCK-B receptors in the rat, rabbit, and monkey. Previous data on other receptors coupled to G-proteins suggest that CCK receptors expressed by trigeminal and DRG neurons are transported both centrally to the spinal cord and peripherally to the innervated tissue and will constitute a target for released CCK at both sites. Possible functions mediated by the CCK receptors expressed by DRG neurons include modulation of DRG neurotransmitter release in both the spinal cord and the innervated tissue, regulation of opiate analgesia, and regulation of the development of morphine tolerance.

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