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Autoradiographical Detection of Cholecystokinin-A Receptors in Primate Brain Using ^{125}I-Bolton Hunter CCK-8 and ^{3}H-MK-329

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***In vitro* autoradiography was performed in order to visualize cholecystokinin-A (CCK-A) receptors in sections of Cynomolgus monkey brain. CCK-A receptors were defined as those which displayed high affinity for the selective non-peptide antagonist MK-329 (L-364,718) and were detected in several regions by selective inhibition of ^{125}I-Bolton Hunter CCK using MK-329 or direct labeling with ^{3}H-MK-329. In the caudal medulla, high densities of CCK-A sites were present in the nucleus tractus solitarius, especially the caudal and medial aspects, and also the dorsal motor nucleus of the vagus.**

CCK-A sites were localized to a number of hypothalamic nuclei such as the supraoptic and paraventricular nuclei, the dorsomedial and infundibular nuclei as well as the neurohypophysis. The mammillary bodies and supramammillary nuclei also contained CCK-A receptor sites. High concentrations of CCK-A receptors were present in the substantia nigra zona compacta and also the ventral tegmental area and may be associated with dopamine cell bodies. Binding of ^{3}H-MK-329 was also detected in parts of the caudate nucleus and ventral putamen.

The detection, by autoradiographical means, of CCK-A receptors throughout the Cynomolgus monkey brain contrasts with similar studies performed using rodents and suggests differences in the density and, perhaps, the importance of CCK-A receptors in the primate as opposed to the rodent. The data suggest the possibility that CCK-A receptors may be involved in a number of important brain functions as diverse as the processing of sensory information from the gut, the regulation of hormone secretion, and the activity of dopamine cell activity.

The peptide cholecystokinin (CCK) is common to both brain and gut (see Vanderhaeghen et al., 1975; Dockray, 1976). Sulfated CCK octapeptide is widely distributed throughout the brain and spinal cord (Vanderhaeghen et al., 1975; Emson et al., 1982; Crawley, 1985), and although its function is unknown, it may act as a neuromodulator or neurotransmitter. This notion is supported by the finding that CCK binding sites, which may represent receptors, are widely distributed throughout the brain (Saito et al., 1980; Van Dijk et al., 1984; Kritzer et al., 1987), and this distribution parallels the presence of CCK-like immunoreactivity. Although these binding sites were originally considered as being a homogeneous population, recent evidence suggests this is an oversimplification.

The possibility that receptors for CCK may exist in more than one form was first proposed by Innis and Snyder (1980) on the basis of differential affinities of CCK-related peptide fragments, measured in binding studies using separate tissues. Thus, desulfated CCK-8 (dCCK) gastrin and the C-terminal tetrapeptide of CCK, CCK-4, discriminated between binding sites in the brain and pancreas: the unsulfated fragments only binding weakly to peripheral receptors. However, these studies provided no evidence for subtypes of binding site within the brain.

Although *in vivo* pharmacological experiments have suggested that CCK-receptor binding sites in brain may well be heterogeneous (Hommer and Skirboll, 1983; Hommer et al., 1985, 1986), the interpretation of these data is complicated by the use of systematic administration of peptides which do not cross the blood–brain barrier. Only more recently have pharmacological data supporting the existence of central CCK receptor subtypes been obtained where the peptides have been administered directly to neurons (Wang et al., 1988).

The development of the non-peptide antagonist, MK-329 (L-364,718) (Chang and Lotti, 1986; Evans et al., 1986) provided a much firmer basis upon which to differentiate CCK-receptor subtypes. This compound possesses much greater selectivity for peripheral or CCK-A receptors than any previously available; blocking ^{125}I-Bolton Hunter binding to pancreatic membranes at subnanomolar concentrations, while only significantly affecting CCK-B receptor binding sites at concentrations in excess of 30 nM (Chang and Lotti, 1986; Hill et al., 1987b). Despite this, early binding studies using homogenates of rodent brain again failed to reveal any heterogeneity of CCK binding sites (Chang and Lotti, 1986).

Moran and colleagues (Moran et al., 1986) provided the first direct autoradiographical evidence for the existence in rat brain of CCK receptors which resembled those found in peripheral tissues. They described binding sites in a number of regions which bound ^{125}I-CCK-33 but had relatively low affinity for desulfated CCK, CCK-4, and gastrin. These observations were confirmed and extended by ourselves using MK-329 and its analog L-365,031 (Hill et al., 1987a, b; Hill and Shaw, 1988). Thus, in the midbrain and caudal medulla, discrete regions were detected in which binding of ^{125}I-Bolton Hunter CCK-8 (^{125}I-BHCCK-8) was selectively displaced by low concentrations of MK-329. In the medulla, the areas showing the highest density

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of MK-329 sensitive sites were the area postrema and medial aspects of the nucleus tractus solitarius (NTS), with CCK-receptor binding sites in the lateral NTS being much less sensitive to the antagonist. In the midbrain, the interpeduncular nucleus (IPN) was most clearly defined in terms of CCK-A sites (Moran et al., 1986; Hill et al., 1987a, 1988a; Hill and Shaw, 1988).

Subsequent studies using different species have shown that CCK-A receptors may not always be localized to identical brain areas. For instance, CCK-A receptors were detected in the region of the IPN in both rat and mouse but were not present in analogous sections of guinea pig brain (Hill et al., 1987b). However, perhaps the most striking difference between species was seen in the spinal cord. In the rat, for instance, CCK-receptors localized to the substantia gelatinosa are of the CCK-B class: binding MK-329 with low affinity and discriminating poorly between sulfated and desulfated CCK (Hill et al., 1988b). By contrast CCK-binding sites in the same region of primate spinal cord are CCK-A sites and show very high affinity for MK-329, which can be used in its tritiated form in autoradiographical experiments (Hill et al., 1988b).

The existence of CCK-A receptors in the spinal cord of the primate made us consider the possibility that may also exist in higher regions of the primate neuraxis, and so a series of experiments was conducted to investigate this possibility.

Materials and Methods

A male Cynomolgus (*Macaca fascicularis*) monkey was used for generation of the autoradiographs presented here, but similar results have since been obtained in 2 other animals. In each case, clear evidence was obtained for the existence of CCK-A sites in the hypothalamus, basal ganglia, mid- and hind brain, and spinal cord. Comparable data has also been obtained in preliminary experiments using tissue from squirrel monkey, rhesus monkey, and human.

The animal was killed by giving an overdose of Nembutal, and the brain and spinal cord were carefully dissected out. The spinal cord was separated from the rest of the brain, which was then divided into 2 parts by a transverse cut just caudal to the substantia nigra which separated the parietal and occipital lobes from the rest of the cortex. The resulting 2 blocks of tissue were then frozen at −70°C.

Serial sections (20 μm) were cut from the blocks using a cryostat, and the sections were thaw-mounted onto gelatin-subbed slides and allowed to dry at room temperature. Once dry, the sections were frozen at −70°C and kept at this temperature until required.

On the day of use, the sections were thawed, dried, and then incubated for 10 min in 10 mM HEPES, pH 7.4. After this, the sections were transferred to polythene vessels containing 20 mM HEPES (pH 6.5), 5 mM $MgCl_2$, 150 mM NaCl, 1 mM EGTA, and 0.025% bacitracin to which was added 50 pM ^{125}I-BHCCK-8 or 200 pM ^{3}H-MK-329 with or without unlabeled test drug. After 60 min (if ^{3}H-MK-329 was used) or 120 min (for ^{125}I-BHCCK), the sections were washed 4 times in ice-cold buffer (6 min each for ^{125}I-BHCCK or 2 min each for ^{3}H-MK-329) and then dipped briefly in distilled water to remove excess salts. The sections were dried under a stream of cold air and were placed against tritium-sensitive film together with radioactive standards. The resulting autoradiographs were then used as negatives for the generation of the photographs shown here. In cases where ^{125}I-BHCCK was used as the radioligand, the level of binding was quantified using a Quantimet 970 image analyzer (Cambridge Instruments) with calibration by means of brain paste standards containing known amounts of ^{125}I. Throughout this series of experiments, nonspecific binding to CCK binding sites was determined using 1 μM sulfated CCK octapeptide and 10 nM MK-329 was used to define CCK-A sites.

Under the experimental conditions described above, only compounds related to CCK and gastrin displace ^{125}I-BHCCK-8 binding. Moreover, the pharmacological profile of the binding sites labeled by ^{125}I-BHCCK corresponds closely to that described for CCK-A and CCK-B receptors in functional studies (Boden and Hill, 1988; Hill and Boden, 1989).

Following autoradiography, sections were stained for acetylcholinesterase activity or with cresyl violet. The stained sections were used in conjunction with the autoradiographs to determine the anatomical localization of the binding sites.

Materials. ^{125}I-BHCCK was obtained from Amersham International U.K. (specific activity, 2000 Ci/mmol). ^{3}H-MK-329 (specific activity, 67.4 Ci/mmol) was synthesized by Dr. A. Rosegay of M.S.D. Laboratories (Rahway, NJ), and unlabeled MK-329 was prepared by M.S.D. Laboratories (West Point, PA). CCK-8 was purchased from Cambridge Research Biochemicals U.K. All other reagents were of analytical grade and were purchased from commercial sources.

Results

Specific CCK receptor binding sites, defined by displacement of ^{125}I-BHCCK by 1 μM sulfated CCK-8, were unevenly distributed throughout the monkey brain. Nonspecific binding represented approximately 40% of total binding and was uniform throughout a section suggesting regional variations represented true differences in receptor densities. Particularly high levels were detected in the cerebral cortex, cerebellum, basal ganglia, nucleus accumbens, and discrete regions of the medulla. Slightly lower levels were detected in the hypothalamic nuclei and the midbrain (Figs. 1, 2, 4, 5). Regional variations in specific ^{125}I-BHCCK binding are shown as color-coded images presented alongside the image of total binding from which they were derived.

CCK-A receptors represented a small proportion of the total CCK receptor binding sites present in brain and were found to be predominantly associated with 3 anatomical regions: the hypothalamus, including the mammillary body, the substantia nigra and its projection fields in the basal ganglia, and the dorsal medulla. Each of these will be considered separately.

Hypothalamus

CCK-A sites were detected throughout the hypothalamus. High concentrations were present in the paraventricular nucleus (PVN) and in the supraoptic nucleus (SON) dorsal to the optic nerve. ^{125}I-BHCCK binding was localized to a crescent formation overlying the optic nerve (Fig. 1*A*). This was also seen when ^{3}H-MK-329 was used as the radioligand (Fig. 3) to selectively label CCK-A receptors. Unfortunately, the sections used with ^{3}H-MK-329 were taken slightly too far anterior to contain the PVN.

Sections made in a progressively more caudal direction from the SON revealed CCK-A sites localized to the margins of the third ventricle and in particular to the dorsomedial nuclei (DMN) of the hypothalamus. This was shown using both ^{3}H-MK-329 and ^{125}I-BHCCK to label the sites selectively (Figs. 1*B*, 3). Specific CCK-A receptor binding was also detected using ^{125}I-BHCCK in the infundibular regions of the hypothalamus and the neurohypophysis (Figs. 1*B*, 2). Displaceable ^{3}H-MK-329

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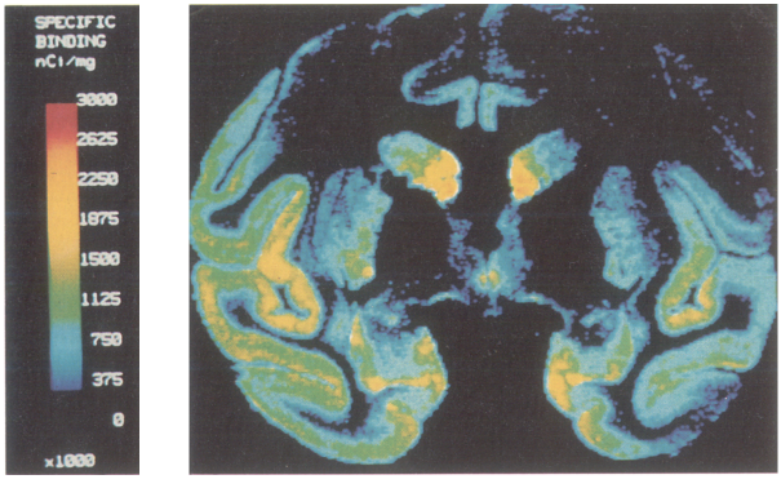
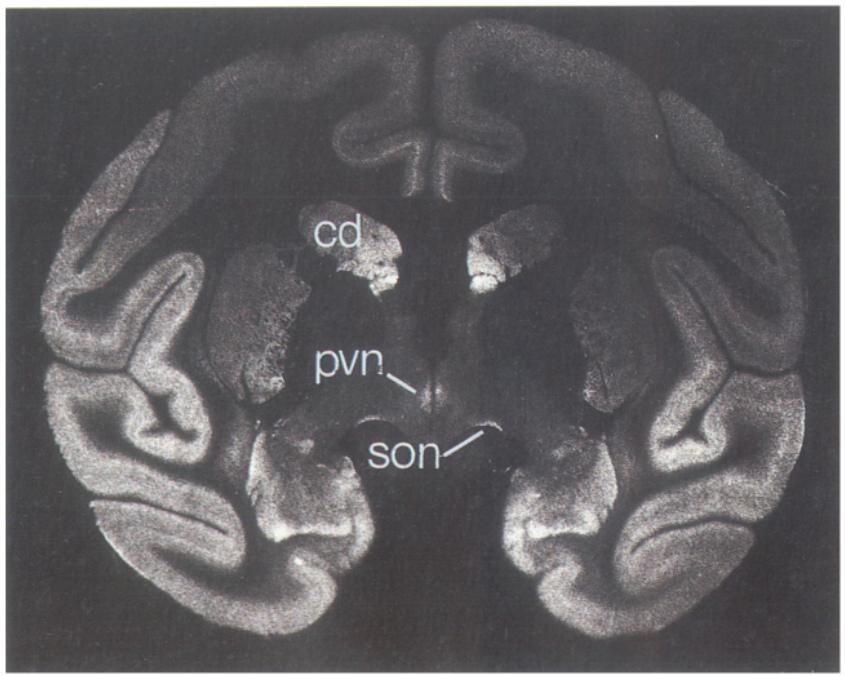
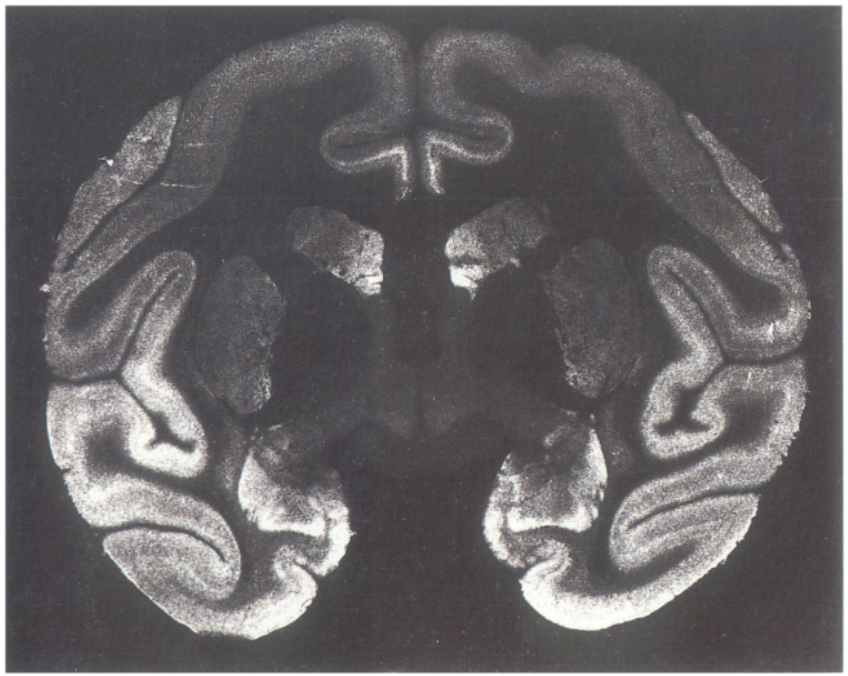
Figure 1. *A* and *B*, Autoradiographs showing ^{125}I-BHCCK binding to sections of Cynomolgus monkey brain and its selective displacement by MK-329. Sections were incubated for 120 min at 20°C with 50 pM ^{125}I-BHCCK either alone or in the presence of 10 nM MK-329 and used to generate the autoradiographs shown here. Color-coded images of specific binding of ^{125}I-BHCCK were generated by a digital subtraction of nonspecific binding (defined by 1 μM CCK) from total binding. MK-329 selectively inhibited binding from CCK sites in the supraoptic (*son*) and paraventricular nucleus of the hypothalamus (*1A*), and from sites in the dorsomedial (*dmn*) and infundibular nucleus of the hypothalamus (*1B*). Note also the small degree of inhibition of ^{125}I-BHCCK binding to the caudate nucleus which occurred in the absence of any clear reduction in binding to sites in the cerebral cortex.

SPECIFIC

A





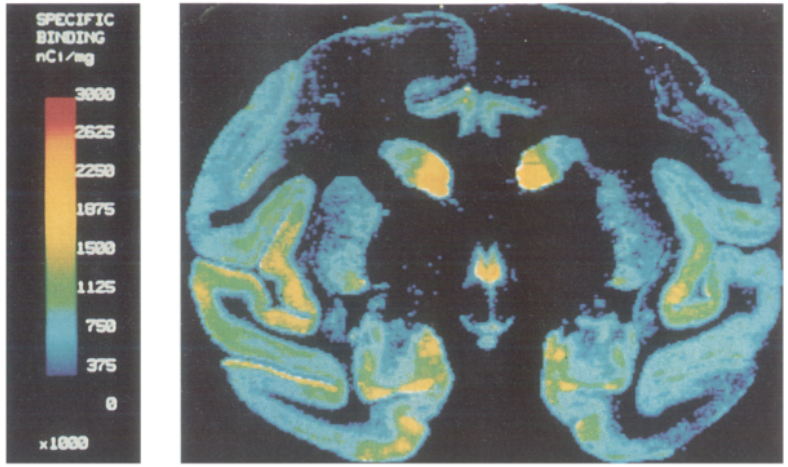


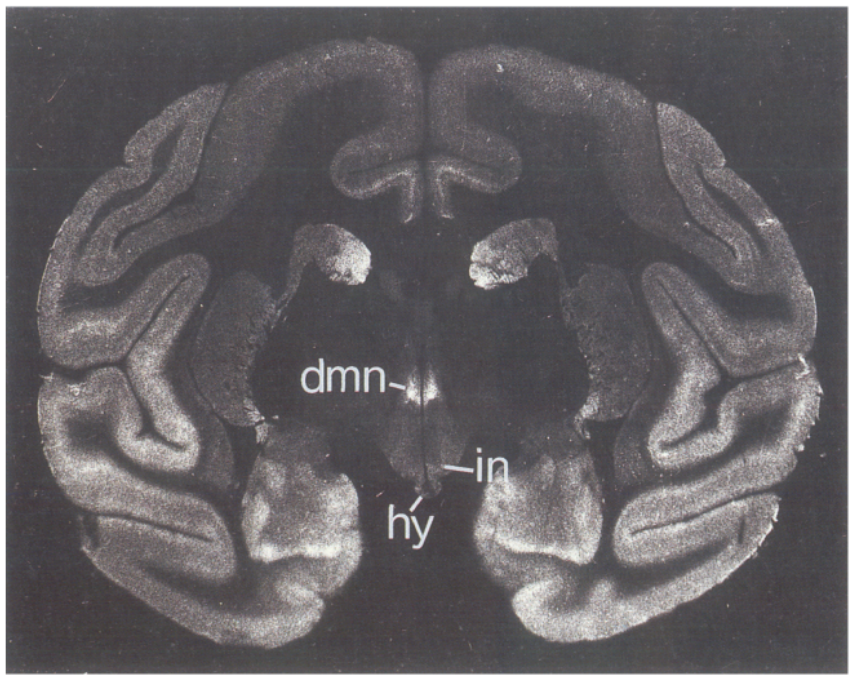
TOTAL

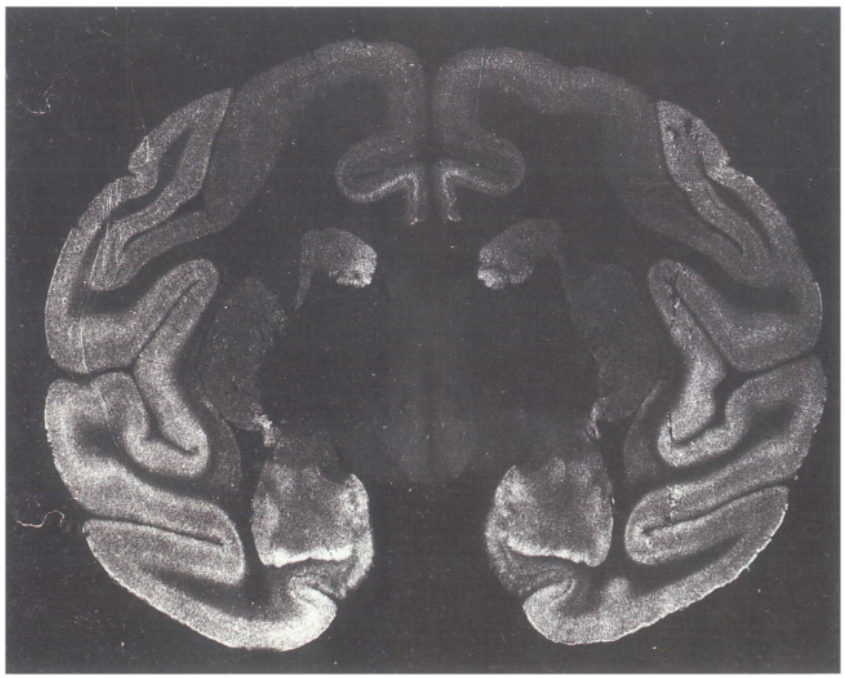
+MK-329

SPECIFIC

B

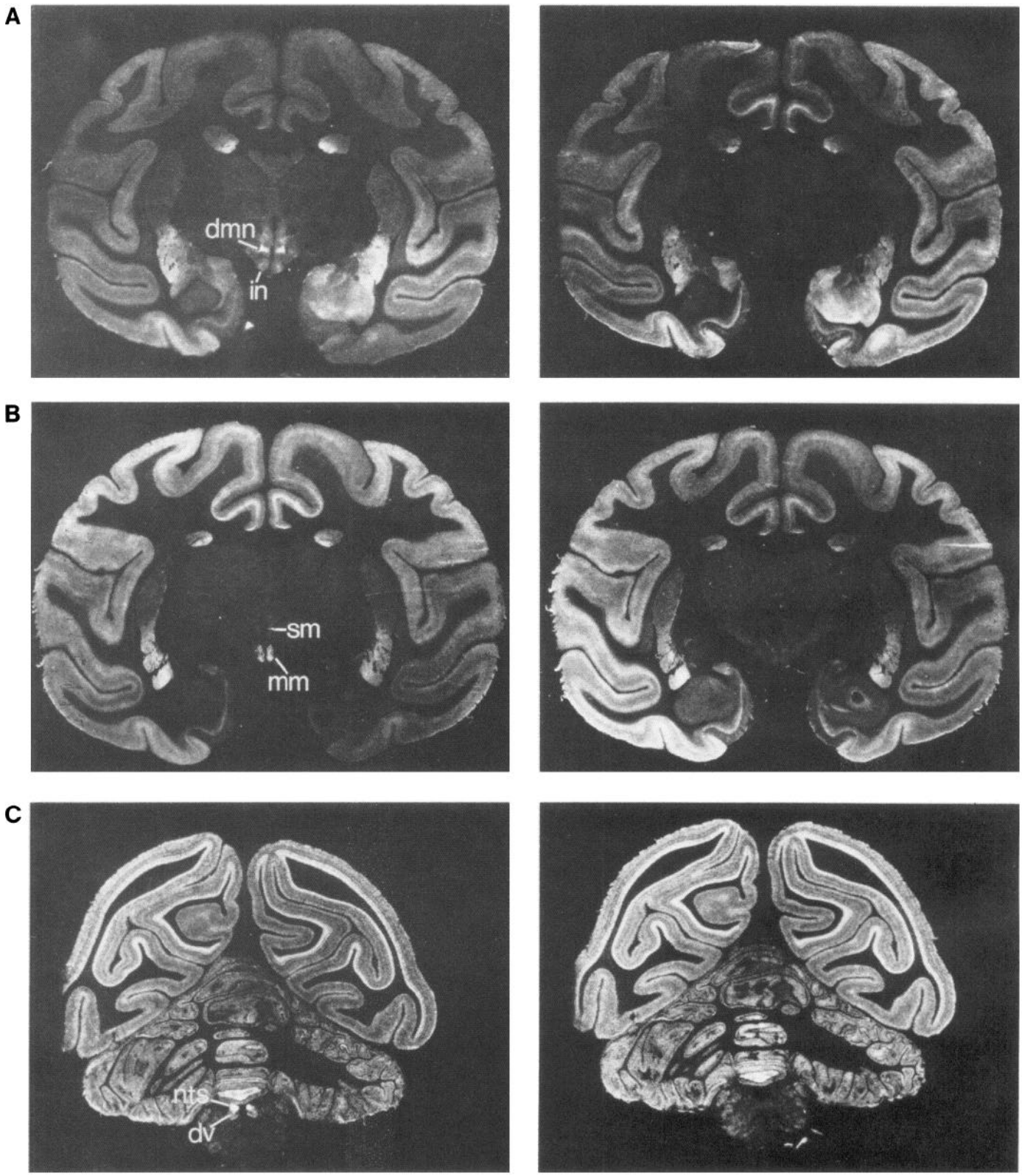






TOTAL

+MK-329


TOTAL
+MK-329
A
dmn
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binding to the neurohypophysis was also detected in sections where it remained attached to the rest of the section (Fig. 3).

Little evidence was found of any specific binding in the ventromedial hypothalamic nucleus. Indeed, the margins of this nucleus could be discerned contrasting against the presence of ^{3}H-MK-329 or ^{125}I-BHCCK binding in the DMN above and the infundibular nucleus below the ventromedial nucleus itself (Figs. 1B, 3).

CCK-A sites were present throughout the mammillary bodies and also in the supermammillary nuclei, although the level of binding in the latter was only just detectable (Figs. 2, 3).

Substantia nigra and basal ganglia

Moderate to high levels of ^{125}I-BHCCK binding were detected in the substantia nigra (Figs. 4, 5), and this was completely inhibited by the presence of MK-329. CCK-A receptors were localized to the pars compacta and in rostral sections, binding was detected as a well-defined band of uniform intensity (Fig. 4). In more caudal sections of the nigra, the localization was rather more diffuse (Fig. 4), perhaps reflecting the presence of CCK-A receptors on dendritic projections into the pars reticulata. Overall, localization of CCK-A binding throughout the nucleus sites closely paralleled the distribution of the large cell bodies of the pars compacta visualized by Nissl and acetylcholinesterase stains (see Francois et al., 1985). The highest density of CCK-A sites in the mesencephalon was found in a region dorsomedial to the substantia nigra and ventral to the red nucleus representing the ventral tegmental area (VTA; Fig. 5). Specific ^{3}H-MK-329 binding to this area was also pronounced (Fig. 5).

During the course of these experiments, it became apparent that ^{125}I-BHCCK binding to regions of the basal ganglia, notably parts of the caudate nucleus and ventral putamen, was partially inhibited by the presence of MK-329, whereas binding to other regions was not (see, for example, Figs. 1, 2, 4, 5). This suggested that these regions may contain a heterogeneous population of both CCK-A and CCK-B receptors. The degree of inhibition of ^{125}I-BHCCK to CCK-A sites in these regions was too small to quantify. However, the possibility that CCK-A receptors may be present in parts of the neostriatum was supported by the detection of significant levels of specific ^{3}H-MK-329 binding to the caudate and putamen (Figs. 3, 5).

Dorsal medulla

Very high densities of CCK-A receptor binding were detected in the dorsal medulla and, in particular, the area postrema and nucleus tractus solitarius (Fig. 2). In fact, CCK-A sites were detected at most levels of the nucleus tractus solitarius extending rostrally from the commissural nucleus, although they were concentrated in the more caudal and medial regions of the nucleus. Slightly lower levels of ^{125}I-BHCCK binding to CCK-A sites were detected in the dorsal motor nucleus of the vagus (Fig. 2).

Discussion

The present data show that several regions of the brain of the Cynomolgus monkey contain CCK receptors of the CCK-A subtype, that is, they show high affinity for the selective CCK-A antagonist MK-329. Until recently, brain CCK receptors were considered as a homogeneous population (Innis and Snyder, 1980; Saito et al., 1980), but autoradiographic experiments in the rat have shown this not to be the case (Moran et al., 1986; Hill et al., 1987a). In these studies, areas of rat brain were found, such as the nucleus tractus solitarius and the area postrema, which contained CCK receptors that resembled peripheral (CCK-A) receptors in terms of their affinity for the agonists desulfated CCK or gastrin and the antagonist MK-329. In the primate CNS, CCK-A sites appear to be more widespread than studies in the rat would have suggested, being found not only in the spinal cord (Hill et al., 1988b) but also throughout the brain.

Medulla and hind brain

The distribution of CCK-A sites in the caudal medulla of the monkey resembled that seen in previous studies using the rat (Moran et al., 1986; Hill et al., 1987a); however, some differences were observed. In the rat, for instance, no evidence was found for the presence of CCK-A sites in the dorsal motor nucleus of the vagus, whereas significant labeling of this structure was seen in the monkey. Furthermore, differences between lateral and medial NTS as to which receptor subtype predominated were seen in the rat, but not the monkey medulla (Hill et al., 1987a). Although CCK receptors were detected throughout the rostrocaudal axis of the monkey NTS, the highest concentration was measured in the caudal and medial portions which receive mainly visceral afferents and, in particular, those from the alimentary tract (Kalia and Mesulam, 1980; Higgins and Schwaber, 1981; Leslie et al., 1982; Carpenter and Sutin, 1983). This pattern was similar to that seen in the rodent. Lesioning studies in the rat suggest that CCK-A sites in the medial NTS are localized, at least in part, on the terminals of vagal afferents which project to the nucleus, as they are reduced in number following either unilateral vagotomy or capsaicin treatment (Ladenheim et al., 1987; Moran and McHugh, 1988). This finding is consistent with data showing axonal flow of CCK receptors towards the terminal regions of the vagus nerve (Zarbin et al., 1981; Moran et al., 1987).

Hypothalamus

CCK-A receptors were widely distributed throughout the hypothalamus and were associated with several distinct nuclei, especially the PVN, SON, and DMN. Moreover, the detection of ^{3}H-MK-329 binding in the neurohypophysis suggests the presence of CCK-A receptors extending along axons to the terminals of these hypothalamic neurons. The presence of high densities of CCK receptors in both the SON and PVN of the

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Figure 2. Localization of CCK-A receptors in the hypothalamus (*A* and *B*) and the medulla (*C*) of the Cynomolgus monkey determined using 50 pM ^{125}I-BHCCK and 10 nM MK-329. CCK-A sites were detected in the dorsomedial hypothalamic nucleus (*dmn*) and the infundibular nucleus (*in*). They were also discretely localized to the mammillary bodies (*mm*), and low levels of MK-329-sensitive binding could just be discerned in the supermammillary nuclei (*sm*). Note that binding of ^{125}I-BHCCK to the medial aspects of the caudate nucleus was also partially reduced by MK-329 (*B*). High densities of CCK-A receptors were localized to the nucleus tractus solitarius (*nts*) and to the dorsal vagal motor nucleus (*dv*) of the medulla (*c*).

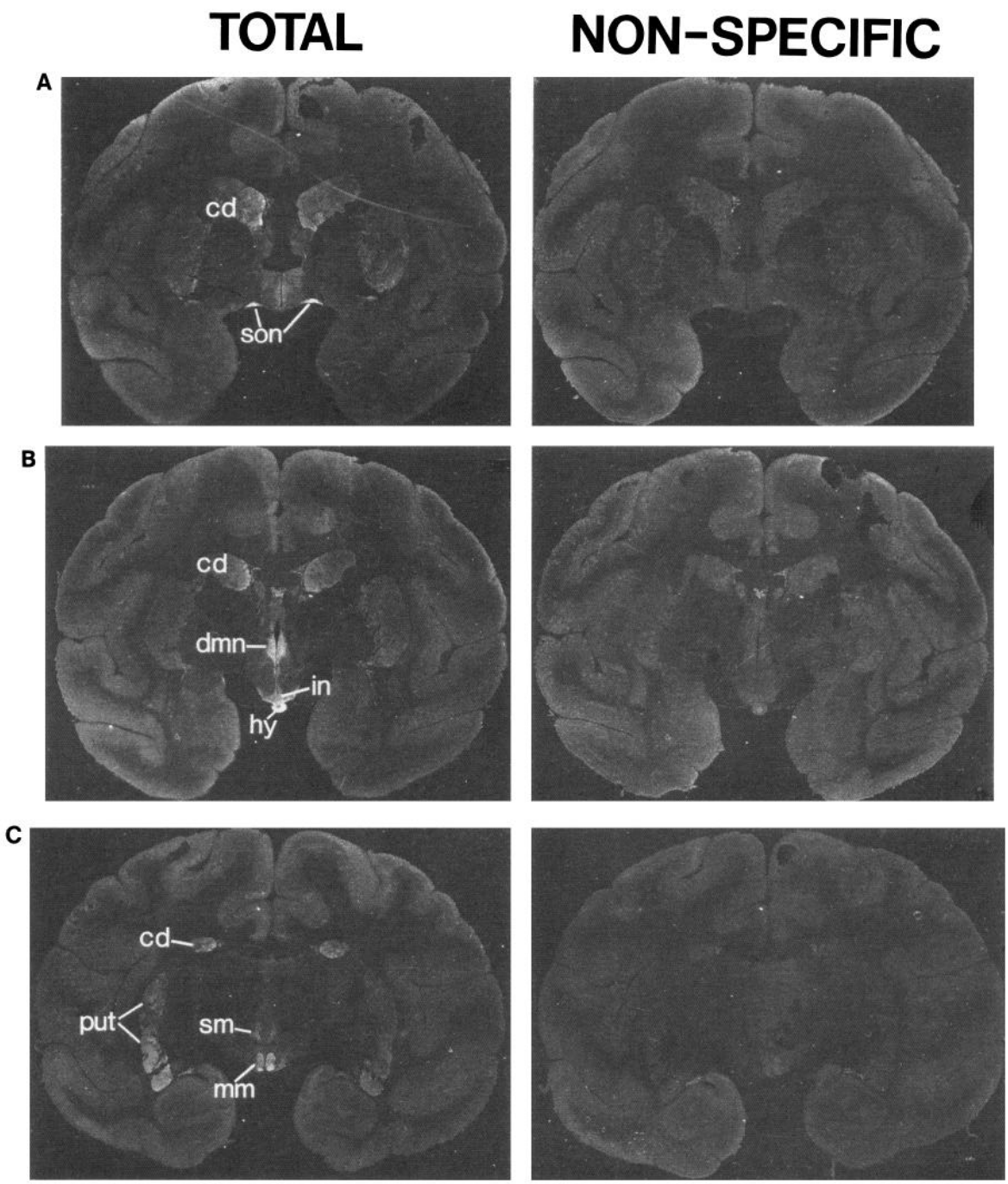

Figure 3. Autoradiographs of ^{3}H-MK-329 binding to different regions of Cynomolgus monkey brain. Adjacent sections were incubated with 200 pM ^{3}H-MK-329 for 60 min at 20°C in the presence or absence of 10 nM unlabeled L-364,718. The sections were then washed 4 times for 2 min each in ice-cold buffer. The dried sections were apposed to tritium-sensitive film for 8 weeks in order to generate the autoradiographs shown here. Displaceable binding of ^{3}H-MK-329 was detected in the supraoptic nucleus (*son*), the dorsomedial hypothalamic nucleus (*dmn*), and the infundibular nucleus (*in*), as well as the mammillary bodies (*mm*), the supermammillary nuclei (*sm*), and the neurohypophysis (*hy*). Specific binding of ^{3}H-MK-329 was also detected in the medial caudate (*cd*) and the ventral putamen (*put*).

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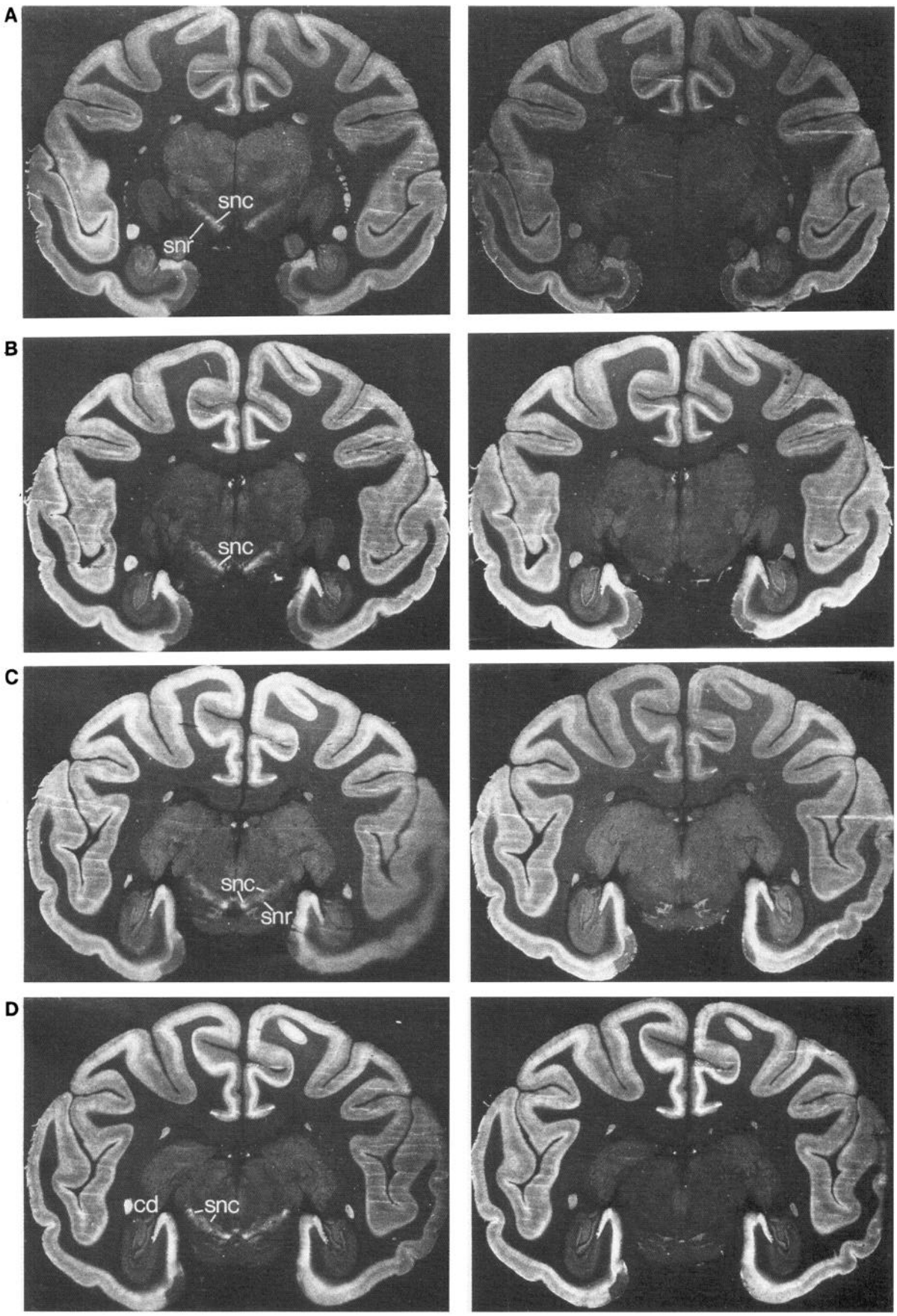
Figure 4. Autoradiographs of ^{125}I-BHCCK binding to sections taken in a progressively caudal direction through the substantia nigra. CCK-A receptors defined by 10 nM MK-329 were present throughout the rostrocaudal axis of the substantia nigra and were concentrated in the zona compacta (*snc*) rather than the zona reticulata (*snr*).


TOTAL
+MK-329
A
snc
snr
B
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C
snc
snr
D
cd
snc

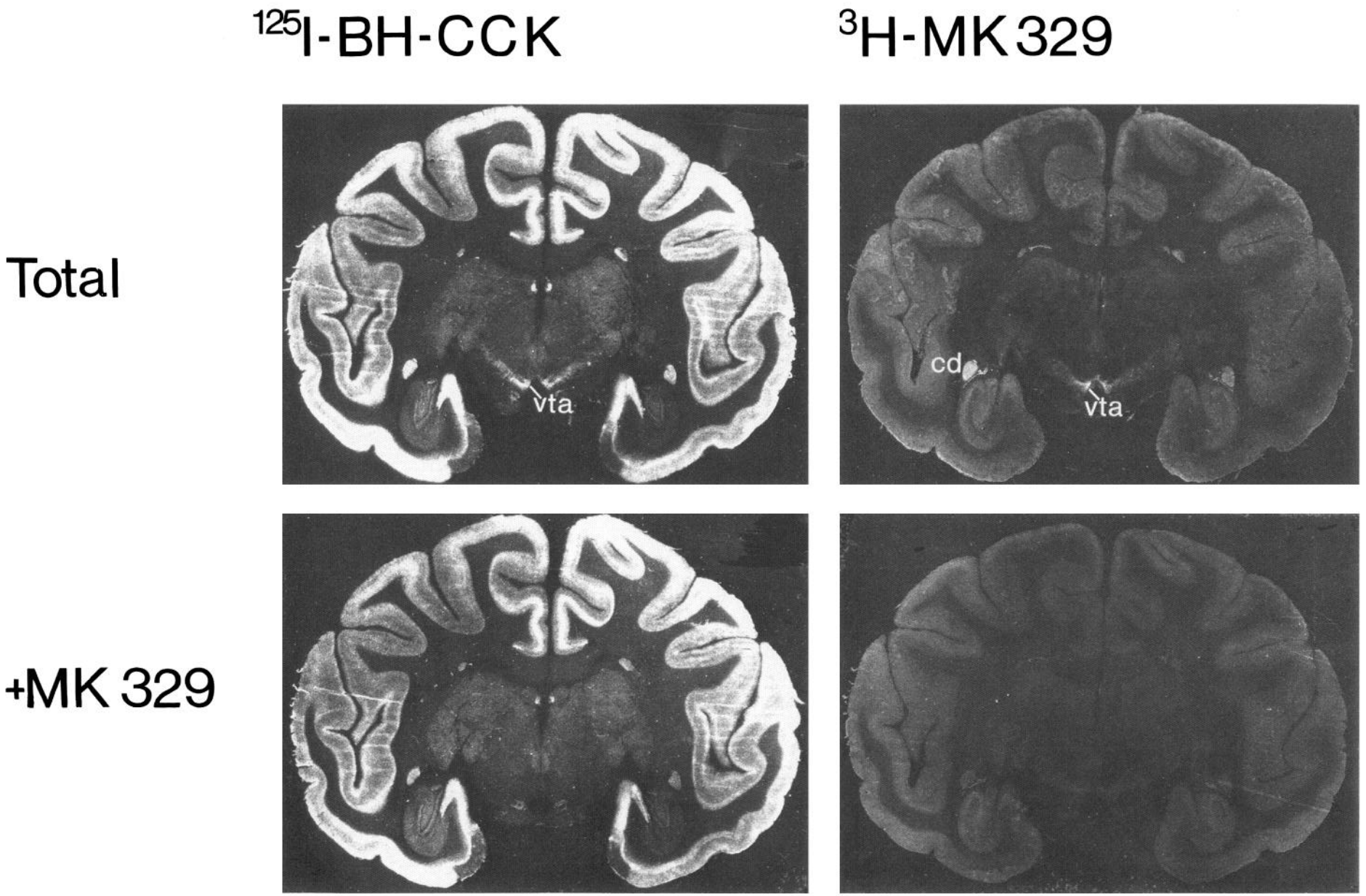

Figure 5. Autoradiographs showing the presence of CCK-A receptors in the substantia nigra (*sn*) and ventral tegmental area (*vta*). Sections were incubated with either 50 pM ^{125}I-BH-CCK or 200 pM ^{3}H-MK-329 in the presence or absence of 10 nM unlabeled MK-329. MK-329 selectively inhibited ^{125}I-BHCCK binding to the zona compacta of the substantia nigra and also partially reduced binding in the caudate nucleus. These same regions were selectively labeled by ^{3}H-MK-329, with specific binding to the ventral tegmental area being particularly clear.

monkey reflects a similar finding in the rat where the distribution of receptors parallels the presence of CCK immunoreactivity (Vanderhaeghen et al., 1981; Vanderhaeghen, 1985; Clark et al., 1987). Furthermore, CCK has been shown to coexist with oxytocin in terminals within the neurohypophysis (Martin et al., 1983).

The possibility of a close functional association between CCK and the activity of the magnocellular neurons of the SON and PVN existing in the primate is supported by experiments in the rat. These have shown marked increases in the density of CCK receptors in the SON and PVN under conditions such as salt loading, which are known to both activate the hypothalamic–pituitary axis and reduce the CCK content of the posterior pituitary (Deschepper et al., 1983; Clark et al., 1987). Although CCK receptors in the rat may differ from those in the monkey, the data suggest that CCK receptors play an important role in the regulation of hormone secretion from magnocellular neurons of the hypothalamus and may be important in pathological states.

Although CCK-A sites were present in relatively high concentrations in the DMN of the hypothalamus, no evidence of CCK receptors of either subtype was found in the ventromedial nucleus. This was rather surprising given the high concentration of CCK-B receptors in the same region of the rat (Akesson et al., 1987; Day et al., 1987; Boden and Hill, 1988). However, considerable species differences between the localization of CCK-A and CCK-B receptors have been reported (Van Dijk et al., 1984; Mantyh and Mantyh, 1985; Williams et al., 1986; Hill et al., 1987b; see also Dietl et al., 1987, for human data), so this may represent yet another example of the phenomenon. An alternative explanation may be that high concentrations of endogenous peptide bound to the receptors in this region prevent labeling by the radioligand, as is the case for somatostatin receptors (Leroux et al., 1988).

The high concentration of CCK-A sites throughout the dorsomedial hypothalamic nucleus and the significant density in the mammillary bodies was another example of the discrete localization of CCK receptor sites with individual nuclei. Although the function of these hypothalamic nuclei is unclear, it is of interest that the DMN represents an area that receives significant dopaminergic input (Carpenter and Sutin, 1983) and may represent another example of an association between dopamine and CCK.

Substantia nigra

The presence of high concentrations of CCK-A receptors in the substantia nigra and VTA was perhaps the most significant finding of the present study. CCK has been shown to coexist with dopamine in neurons of the substantia nigra and the VTA in a number of species (Hökfelt et al., 1980a, b, 1985; Seroogy et al., 1988), and a variety of evidence suggests that CCK can influence the activity of dopamine neurons in a complex manner. Data from *in vivo* and *in vitro* experiments performed using the rat have shown that CCK can both excite neurons in the substantia nigra and enhance the inhibitory effect of apomorphine and dopamine itself (Hommer and Skirboll, 1983; Hommer et al., 1985, 1986; Brodie and Dunwiddie, 1987; Freeman and Chiodo, 1988). However, it is unclear whether these effects of CCK are mediated via CCK-A or CCK-B receptors. *In vivo* studies suggest at least part of the action of CCK, notably the ability to enhance the inhibitory effects of dopamine agonists, is mediated by way of CCK-B receptors as this action is selectively induced by desulfated CCK and CCK-4, both of which show only weak activity at CCK-A sites (Innis and Snyder, 1980; Saito et al., 1980; Moran et al., 1986; Hill et al., 1988b). Nonetheless, the presence of CCK-A receptors in the rat substantia nigra is suggested by the observation that sulfated CCK, but not desulfated CCK or CCK-4 can excite dopamine cells (Hommer et al., 1986; Freeman and Chiodo, 1988). Moreover, studies in our own laboratories have shown that the excitation of nigral neurons by sulfated CCK-8 measured *in vitro* is blocked by low concentrations of MK-329, again indicating the presence of CCK-A receptors (W. Graham, unpublished observations).

CCK-A receptors and dopamine cell bodies

Observations such as those described above clearly provide support for the presence of CCK receptors on dopamine cell bodies, and we have some preliminary evidence that this is also true in the primate. Thus, binding of ^{3}H-MK-329 to sections of substantia nigra taken from monkeys which had received a unilateral infusion of MPTP (which selectively destroys dopamine cell bodies in the zona compacta) was markedly reduced when compared with the contralateral unlesioned side, thereby suggesting that CCK-A receptors are normally present on the dopamine cell bodies.

Basal ganglia

As receptors present on cell bodies also extend along the axon to the neuronal terminals (for example, CCK receptors on the vagus), it was perhaps not surprising that ^{3}H-MK-329 binding was found in parts of the caudate nucleus and putamen, possibly representing binding to presynaptic sites on neurons having their origin in the substantia nigra. Unfortunately, ^{3}H-MK-329 binding was not measured in the nucleus accumbens, where many of the neurons originating in the VTA terminate. Moreover, the very great density of MK-329-insensitive CCK binding sites in the accumbens made it impossible to measure any small degree of inhibition by MK-329. However, given the high density of CCK-A sites within the VTA, their presence on nerve terminals in the nucleus accumbens might be expected. In fact, such a possibility would be consistent with *in vitro* experiments in the rat showing the facilitatory effects of CCK on dopamine release from the nucleus accumbens are blocked by selective CCK-A receptor antagonists, including MK-329 (Vickroy et al., 1988). Experiments are currently in progress using tissues from the basal ganglia and nucleus accumbens of MPTP-lesioned monkeys, in an attempt to answer the question of where CCK-A receptors in these structures are localized.

In conclusion, the present work shows, for the first time, that the primate brain contains a heterogeneous population of CCK receptors and that, in particular, receptors of the CCK-A subtype are associated with discrete anatomical systems including dopaminergic neurons. The detection of relatively high levels of CCK-A sites throughout the monkey brain by autoradiography contrasts with the results of previous studies in the rat (Moran et al., 1986; Hill et al., 1987a) and raises the question of why CCK-A sites are not readily visualized in the rat by autoradiography. One possibility, of course, is that they are not present, but this seems unlikely as a number of laboratories have obtained evidence from pharmacological studies for their existence in the hypothalamus, the nucleus accumbens, and the substantia nigra (Vickroy et al., 1988; Wang et al., 1988; Hill and Boden, 1989). A second possibility is that CCK-A receptors in the rat have a lower affinity for ^{125}I-BHCCK, and thus the low ligand concentrations usually employed are too low for a significant

proportion of CCK sites to be labeled. Alternatively, CCK-A sites may be present in much lower concentrations than the more ubiquitous CCK-B receptor, which prevents detection by normal autoradiographic means.

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