Distribution of serotonin 5-HT$_{1C}$ receptor mRNA in adult rat brain

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Based on in situ hybridization histochemistry (ISHH), we describe the anatomical distribution of the serotonin 5-HT$_{1C}$ receptor mRNA. In addition to the very high levels in epithelial cells of the choroid plexus, 5-HT$_{1C}$ receptor mRNA is found throughout the limbic system, in catecholaminergic cells and in serotonergic neurons. Receptor transcripts are also present in the hypothalamus, numerous motor nuclei and the subthalamus. Our results correlate well with serotonin (5-HT) innervation and receptor binding. Receptor mRNA is present in many brain structures in addition to regions previously shown to have 5-HT$_{1C}$ receptor binding. The distribution of this receptor mRNA suggests that the 5-HT$_{1C}$ receptor may mediate a number of the central effects of 5-HT.

Oligonucleotide; Hybridization

1. INTRODUCTION

Serotonergic innervation is widespread through-out the central nervous system (CNS) and the periphery. Serotonin has been implicated in smooth muscle contraction, nociception, appetite, thermoregulation, sleep, sexual behavior, memory, anxiety, depression and hallucinogenic behavior [1–3]. In addition to its direct effects as a neuro-transmitter, serotonin autoregulates 5-HT neu-rons, interacts with other neurotransmitter systems and facilitates motor and sensory neurotransmis-sion [4,5]. Recently, mitogenic effects of 5-HT have also been demonstrated [6,7].

Several receptors and binding sites for serotonin have been defined on the basis of pharmacological and physiological criteria [8]. Following the initial

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Abbreviations: 5-HT, 5-hydroxytryptamine, serotonin; ISHH, in situ hybridization histochemistry; CNS, central nervous system

classification of serotonin-binding sites into 5-HT$_1$ and 5-HT$_2$ [9], at least four subtypes of the 5-HT$_1$ sites, denoted 1A–D, have been characterized in the mammalian brain. In addition, 5-HT$_3$ [10] receptors have been described in rat brain. Altera-tions in serotonin receptor levels have been de-scribed in relation to depression [11,12], in normal aging processes [13–15] and in some [16] but not all [17] suicide victims. Furthermore, changes in 5-HT receptor levels have also been associated with Alzheimer's disease [18,19], Huntington's chorea [20] and schizophrenia [21]. However, the con-tribution of each receptor subtype to normal and disease processes is not understood.

The 5-HT$_{1C}$-binding site was first characterized in pig [22] and rat [23] choroid plexus. Present at extremely high densities on the epithelial cells of the choroid plexus, the 5-HT$_{1C}$-binding site is a functional receptor which mediates phospho-inositide turnover in both rat [24] and pig [25]. Al-though the 5-HT$_{1C}$ receptor may regulate cerebro-spinal fluid production by the choroid plexus [26], the physiological role of this receptor in the brain is not known. Considerably lower levels of binding

sites have been identified in regions of human and rat brain [27,28] than in the choroid plexus.

Recently, the structure of the 5-HT$_{1C}$ receptor has been deduced by molecular cloning of cDNAs from rat [29] and mouse [25]. Based on these nucleic acid sequences, we have designed two oligonucleotide probes for in situ hybridization histochemistry (ISHH). Here, we describe the distribution of neurons containing this serotonin receptor and correlate these results with serotonergic innervation, 5-HT$_{1C}$ receptor binding studies and physiological processes influenced by serotonin. Our data suggest that the 5-HT$_{1C}$ receptor may mediate a number of the central effects of serotonin.

2. MATERIALS AND METHODS

2.1. Oligonucleotide probes

The deduced amino acid sequence of the serotonin 5-HT$_{1C}$ receptor predicts seven transmembrane segments with structural similarity to other members of the G-protein-coupled receptor family. At the amino acid level, the transmembrane regions are most highly conserved among family members, while the amino- and carboxy-termini and the large third cytoplasmic loop are poorly conserved. In order to design probes specific for the 5-HT$_{1C}$ receptor, sequences for oligonucleotides were chosen from the carboxy-terminus (denoted 3A) and the third cytoplasmic loop (termed 2B). Avoiding homology to other known receptors, especially the 5-HT$_2$ receptor subtype [30], two 48-base oligonucleotides (2B, 5'-ATTAGCCAGTTCCTCCTC-GGTGTGACCTCGAAGTAACATCAGAGTTTG-3'; 3A, 5'-GAGCTCCCTCCCAGACAAAGCAGTGGCAGCAACCCT-AGGAATCTGTCG-3') were prepared using an Applied Biosystems DNA synthesizer. Probes for ISHH were A-tailed for 5 min at 37°C as described [31]. Specific activities were 8 × 10^5 cpm/pmol for probe 2B and 1 × 10^6 cpm/pmol for probe 3A.

2.2. In situ hybridization histochemistry

Sections of fresh-frozen adult rat brains (male Sprague-Dawley) were cut thaw-mounted onto gelatin-coated slides and stored at −80°C. Prior to hybridization, sections were warmed to room temperature, fixed in 4% formaldehyde then dehydrated as in [32]. 4 × 10^5 cpm ^{35}S-labeled probe were applied to each section in hybridization buffer [50% formamide/0.6 M NaCl/0.06 M sodium citrate/50 mM sodium phosphate (pH 6.5)/50 mM dithiothreitol/0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin/10% dextran sulfate/250 µg/ml yeast tRNA/500 µg/ml sheared single-stranded salmon sperm DNA]. Hybridizations were performed for 24 h at 37°C. Sections were washed four times for 15 min at 40°C in 50% formamide/0.3 M NaCl/0.3 M sodium citrate and twice for 1 h at room temperature in 0.15 M NaCl/0.015 M sodium citrate. Sections were opposed to Kodak XAR film for 2.5 days, then dipped in Kodak NTB3 nuclear emulsion (1:1 with water).

After exposure at 4°C for 13 days, sections were developed using Kodak D-19 developer (1:1 with water), fixed and counterstained with 0.2% toluidine blue.

3. RESULTS

Probes specific for the carboxy-terminus (3A) and the third cytoplasmic loop (2B) of the 5-HT$_{1C}$ receptor hybridize to identical cells in adjacent thin sections (4 µm) from septal areas (fig.1) as well as brainstem and hippocampus (not shown). In addition, RNA blot analysis of poly (A$^+$) RNA from choroid plexus, cortex and hippocampus reveals a single hybridizing band of about 5.2 kb (not shown) using probe 3A. Taken together, these data indicate that probes 2B and 3A specifically hybridize to a single mRNA for the serotonin 5-HT$_{1c}$ receptor.

In addition to the choroid plexus, serotonin receptor mRNA is present in many neuronal cell bodies throughout the rat brain (fig.2). A detailed description follows.

3.1. Telencephalon

Among rhinencephalic structures, the olfactory bulb, olfactory nuclei (fig.2, panel 1) and olfactory tubercle (panels 1–3) express intermediate levels of mRNA. The nucleus of the diagonal tract (panel 2) also contains intermediate levels of transcripts. Within the hippocampus (panels 9–12), the dentate gyrus, subiculum and layers CA2 and CA3 (fig.3D) show high levels of mRNA while layer CA1 has lower levels. Whereas the frontopolar cortex (panel 1) contains little hybridization, the internal layers of the cingulate cortex (panels 6–10) and pyriform cortex (panels 2–5) contain intermediate amounts. The regions of the basal ganglia (panels 3–6) vary greatly in their expression of serotonin 5-HT$_{1C}$ receptor mRNA. As seen in panel 2, the nucleus accumbens has intermediate levels of labeling while the endopyriform nucleus (panel 6) contains high levels of grains and the claustrum (panel 6) has intermediate levels. Both the caudate-putamen (panel 3–6) and globus pallidus (panels 3–6) contain scattered cells that hybridize to the probe. The subfornical organ has a high level of transcripts while in the septum, the dorsal and lateral nuclei have intermediate amounts, and the medial and triangular nuclei have low amounts of mRNA (panels 3–5). All nuclei of the amygdala (panels 7,8) show at
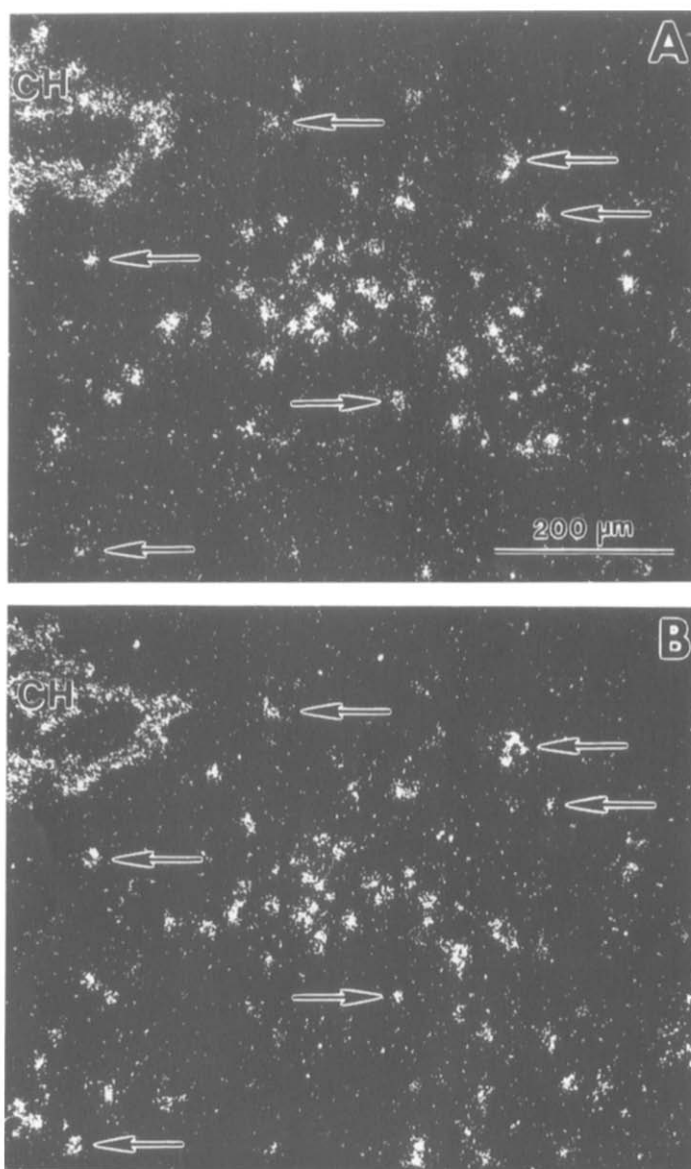
Fig.1. Two oligonucleotide probes for serotonin 5-HT$_{1C}$ receptor mRNA identify the same cells in serial rat brain sections. Photomicrograph of adjacent coronal sections (4 μm) from adult rat brain hybridized with either (A) probe 2B or (B) probe 3A as described. Arrows indicate several of the numerous cells in the septal area which are labeled by both probes. The choroid plexus (CH) is shown for orientation.

least low levels of receptor mRNA; cells of the central and basal (fig.3G) nuclei have intermediate to high levels of hybridization. In addition, the bed nucleus of the stria terminalis (panel 4) has intermediate levels of hybridization.

3.2. Diencephalon

The thalamus (panels 5–10) contains in-termediate levels of labeling in the periventricular and centromedial nuclei as well as the reuniens nucleus. Intermediate levels of labeling are found throughout the reticular formation. The lateral habenula (panels 8,9 and fig.3F) shows high levels of transcripts as seen by Julius et al. [29]. However, low levels predominantly in a crescent-shaped region of the lateral portion of the medial habenula

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are also observed. Both the lateral (panel 10) and medial (panels 11,12) geniculate bodies express intermediate levels of serotonin 5-HT_{1c} receptor mRNA. In the subthalamus (panels 7–10), the zona incerta has intermediate levels of hybridization, but the entopeduncular nucleus (fig.3K) contains high levels of receptor mRNA.

High levels of mRNA transcripts are found in numerous nuclei throughout the hypothalamus. Within the preoptic region (panel 3), the medial and lateral preoptic nuclei show low levels of labeling. The median forebrain bundle (panels 5–10 and fig.3I), the mammillary body and the anterior hypothalamic nuclei (panels 4,5) produce intermediate levels of receptor mRNA. Similar amounts of labeling are observed in the region of the parvocellular CRF-producing cells of the paraventricular nucleus (panel 6 and fig.3J) and in the magnocellular hypothalamic neurons. The ventromedial region of the suprachiasmatic nucleus, most likely the vasopressin-producing cells, shows intermediate labeling (panels 4,5). The dorsomedial nucleus (panel 6) expresses high levels of serotonin 5-HT_{1c} receptor mRNA.

3.3. Mesencephalon

Within the mesencephalon (panels 11–13), numerous regions have receptor mRNA ranging from low to very high levels. The ventral tegmental area (panel 11) and the periaqueductal grey (panels 11,12) show low levels. Within the oculomotor nucleus complex, the Edinger-Westphal nucleus (panel 12) contains intermediate levels of mRNA. Of the most anterior raphe nuclei, the dorsal raphe (panels 12,13) has intermediate levels while the linear raphe nucleus (panels 11,12 and fig.3E) has high levels of mRNA. The interpeduncular nucleus (panels 11,12) which borders the linear raphe nucleus contain only low levels of hybridization. The red nucleus (panel 12) and the cuneiform

nucleus (panel 13) have low levels of transcripts. Both the inferior (panel 13) and superior (panel 12) colliculi express intermediate levels of mRNA. Intermediate densities of mRNA are found in the dopamine-rich substantia nigra (panel 11 and fig.3A) as well as the catecholaminergic nuclei such as A7 and A8 (panels 11–13 and fig.3B).

3.4. Pons

Several regions of the pons express low to intermediate levels of serotonin 5-HT_{1c} receptor mRNA. The pontine nuclei (panel 12) and the superior olive (panel 14) show low amounts of hybridization. Other regions which have intermediate levels of transcripts include the pontine raphe nucleus (panel 13), locus coeruleus (panel 14), the dorsal and ventral parabrachial nuclei (panels 14–16), and the trigeminal motor nucleus (panels 15,16). Like the midbrain, the reticular formation of the pons contains numerous cells which express this serotonin receptor mRNA.

3.5. Cerebellum

In general, the cerebellum (panels 14–17) has the least number of transcripts of the major structures of the brain. In the cerebellar cortex, low levels of mRNA are found in a few scattered granule cells. The cerebellar nuclei have intermediate levels of transcripts.

3.6. Medulla oblongata

The cochlear and vestibular nuclei (panels 15,16) produce low levels of mRNA. The three raphe nuclei of the medulla oblongata, raphe obscurus (not shown), raphe pallidus (not shown) and raphe magnus (panels 14–16 and fig.3H), have intermediate to high levels of receptor transcripts. As in the pons and midbrain, reticular formation in the medulla shows intermediate levels of mRNA with the majority of hybridization in the lateral reticular

Fig.2. Localization of serotonin 5-HT_{1c} receptor mRNA throughout adult rat brain. Low-magnification autoradiograms of 12-μm coronal sections (cut at 200-μm intervals) hybridized with probe 3A as described. Sections are arranged in rostral to caudal order (panels 1–18) with schematic anatomical drawings for orientation. Choroid plexus is identified in several panels by arrowheads. Key for identified structures: A, amygdala; A7, A8, midbrain catecholaminergic cell groups; B, bed nucleur of the stria terminalis; c, central canal; C, cingulate cortex; CA, caudate nucleus; Cer, cerebellum; D, dorsomedial hypothalamic nucleus; E, endopyriform nucleus; EP, endopeduncular nucleus; H, habenula; Hi, hippocampus; IC, inferior collicle; L, lateral geniculate body; lr, linear raphe nucleus; M, medial geniculate body; MF, median forebrain bundle; nc, commisural part of solitary tract; nts, nucleus of the solitary tract; o, olfactory bulb; ot, olfactory tubercle; P, paraventricular nucleus; PO, preoptic area; RM, raphe magnus; S, septum; SC, superior collicle; T, thalamus; ts, nucleus of the spinal trigeminal tract; V, vestibular nuclei; Z, zona incerta.
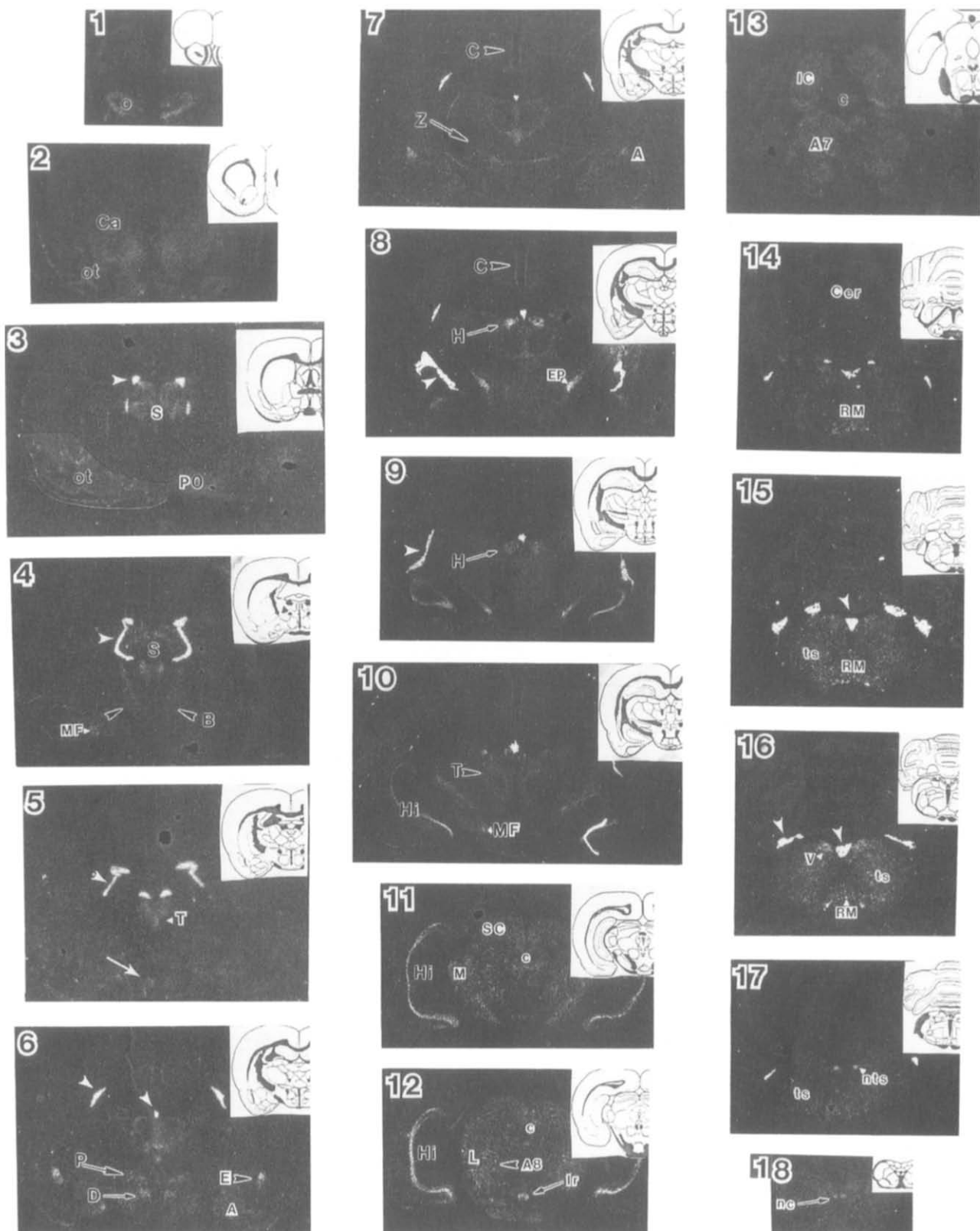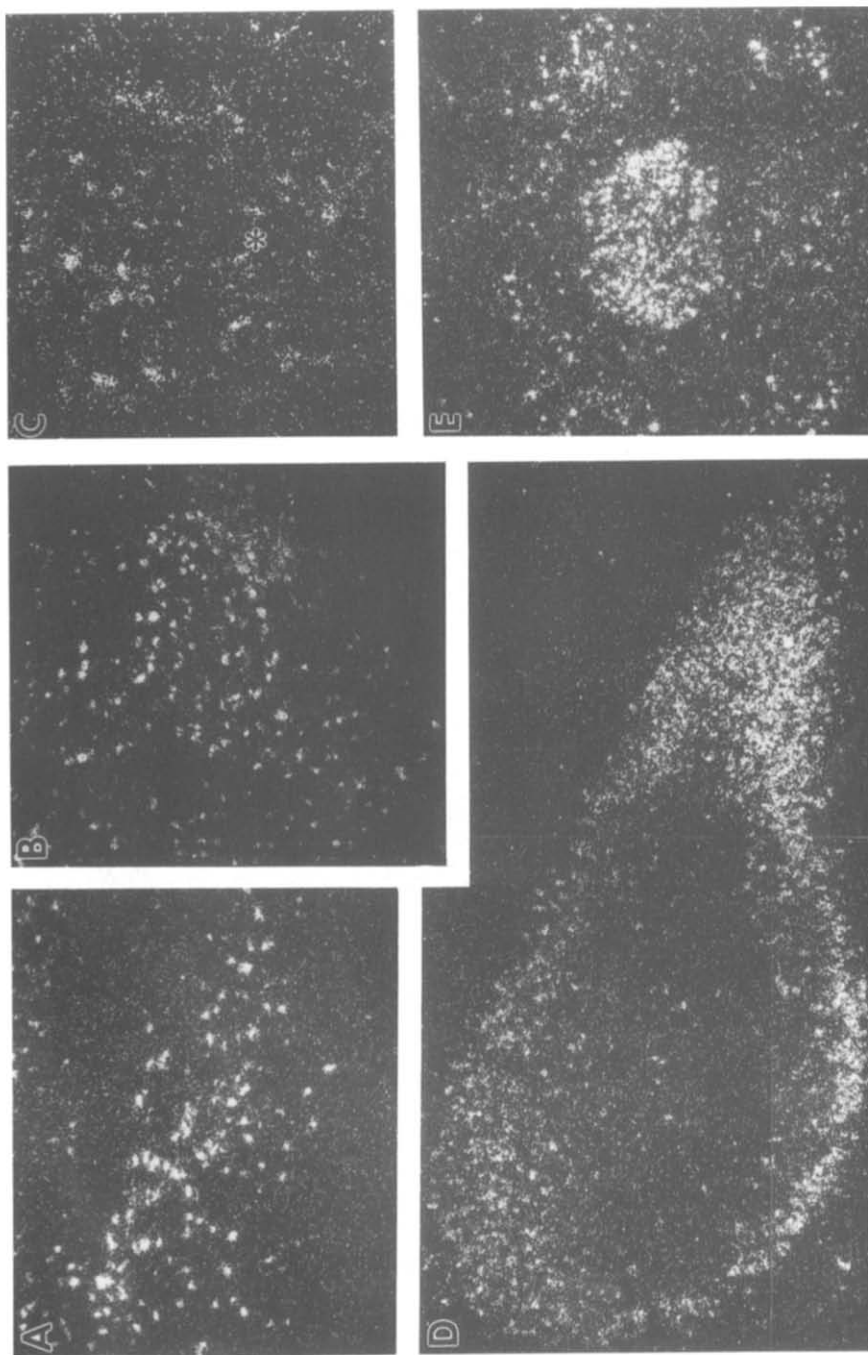
Fig.3. The serotonin 5-HT$_{1C}$ receptor mRNA is expressed in choroid plexus epithelial cells and in neuronal cell bodies. Photomicrographs of selected regions shown in fig.2: (A) substantia nigra, (B) mesencephalon A7 CA cell group area, (C) spinal cord: commissural NTS and motor nucleus of XII nerve, (D) hippocampus CA3 region, (E) linear raphe nucleus (mesencephalon) and interpeduncular nucleus, (F) habenula, (G) basal amygdaloid nucleus, (H) raphe magnus, (I) lateral hypothalamus, (J) PVN, (K) subthalamic (entopeduncular) nucleus. Panels: C, (∗) central canal; F,G, (arrows) choroid plexus; J, (arrowheads) third ventricle; H, (p) pyramidal tract. Magnification, 60×.
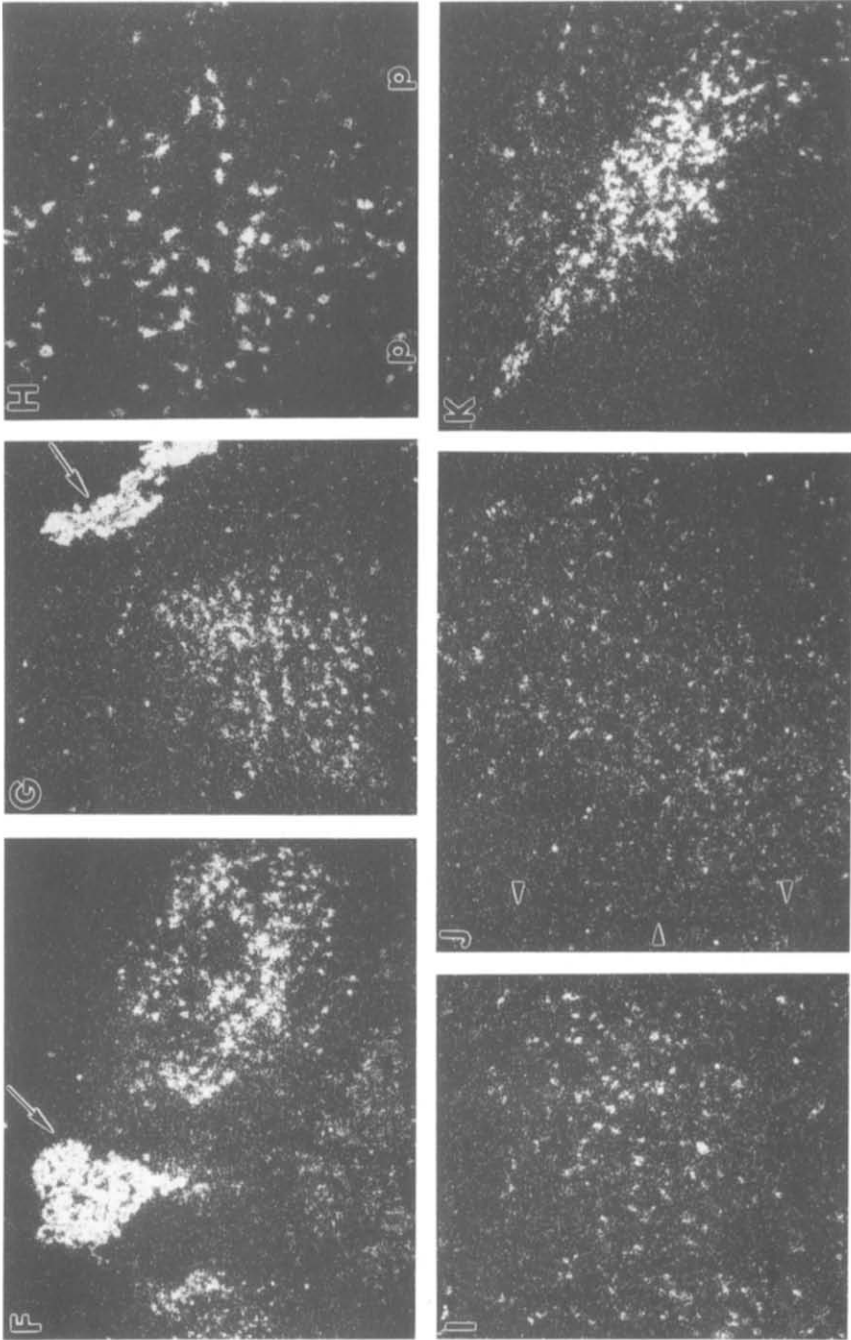
nucleus (panels 16,17). The nuclei of the solitary tract (panels 16–18) have intermediate levels of hybridization with highest amounts of mRNA in the commissural nucleus (panel 18 and fig.3C). The neurons of the brainstem motor nuclei express intermediate to high levels of the receptor mRNA (panels 14–18 and fig.3C).

4. DISCUSSION

The serotonin 5-HT_{1C} receptor is expressed in choroid plexus epithelial cells and numerous neurons throughout the CNS (figs.2,3). As demonstrated by immunocytochemistry, the majority of serotonergic cell bodies are concentrated in the raphe nuclei and innervate nearly all regions of the CNS [33,34]. There is particularly dense innervation of the limbic system including the septal area and amygdala in addition to median forebrain bundle, thalamus, hypothalamus, interpeduncular nucleus, the solitary tract nucleus, locus coeruleus, and the lateral reticular formation. Localization of serotonin 5-HT_{1C} receptor mRNA in rat brain by ISHH correlates very well with serotonergic innervation. That is, 5-HT_{1C} receptor mRNA is not present in areas which do not receive 5-HT innervation. Conversely, not all areas receiving 5-HT innervation contain 5-HT_{1C} receptor mRNA. For instance, layer V [35] of cortex shows 5-HT immunoreactivity as well as post-synaptic 5-HT_2 receptors, but our probes show very little hydridization in this region. This observation further confirms the specificity of these probes for 5-HT_{1C} receptor mRNA. In addition, electrophysiological response to microiontophoresis of serotonin have been recorded from cells in a number of brain regions which both receive serotonin innervation and express 3-HT_{1C} receptor mRNA including hippocampus, amygdala, septum, olfactory bulb, hypothalamus, thalamus, striatum, cuneate nucleus, dorsal lateral geniculate, superior colliculus, dorsal raphe nucleus and reticular formation neurons [33].

Results from in situ hybridization histochemistry were also consistent with analysis of RNA extracted from brain regions [29] showing the highest levels of RNA in the choroid plexus and lower levels in basal ganglia, pons-medulla, hippocampus and hypothalamus. Very little or no 5-HT_{1C} receptor mRNA was detected in cortex, olfactory bulb

and cerebellum by RNA blot analysis [29], a method that is less sensitive than ISHH. While the cortex, olfactory bulb, olfactory tubercle and cerebellum are not devoid of 5-HT_{1C} receptor mRNA, these regions seem to contain populations of cells with little or no mRNA.

The distribution of serotonin 5-HT_{1C} receptors has been characterized by receptor autoradiography [27,28]. The choroid plexus has the highest level of specific binding while several other regions have at least 10-fold lower receptor site densities. Although receptor mRNA is co-localized in most of the regions which also display specific binding, many more areas of the brain express 5-HT_{1C} receptor transcripts than display receptor binding. For instance, the suprachiasmatic nucleus, septum and dentate gyrus (figs.2,3) express high levels of receptor mRNA, but little or no specific binding has been shown in these regions. The increased sensitivity and cellular resolution of ISHH using ^{35}S-labeled probes as compared to ^3H-radioligands may account for these differences. However, mRNA levels need not be proportional to receptor number and receptor protein may be present in structures distant from the cell body transcribing the specific mRNA.

A role for serotonin has been implicated in mood, behavior and hallucinogenic effects [1–3], functions generally associated with the limbic system. With the exception of the choroid plexus, the highest levels of 5-HT_{1C} receptor mRNA are in the limbic structures: hippocampus, septum, amygdala, olfactory nuclei, endopyriform nuclei, cingulate cortex, pyriform cortex. Lysergic acid diethylamide (LSD) has high affinity for 5-HT_{1C} receptors and acts as a partial agonist to stimulate phosphoinositide turnover in choroid plexus [25]. The presence of high levels of receptor mRNA in limbic structures is consistent with the suggestion that the 5-HT_{1C} receptor may mediate responses to psychotropic drugs such as LSD. In addition, 5-HT_{1C} receptors may play a role in 5-HT-associated affective disorders [1,11,12,16,21].

Serotonin has been shown to influence hypothalamic associated functions such as sleep, appetite, thermoregulation, sexual behavior and neuroendocrine functions [1]. The presence of high levels of 5-HT_{1C} receptor mRNA in the medial parvocellular region of the paraventricular nucleus (PVN) implies a role for this receptor in neuroen-

docrine regulation and perhaps stress. Serotonergic neurons in the raphe discharge at a slow rhythmic rate, suggestive of a pacemaker function in the CNS. In addition, the heavy serotonergic innervation and high levels of receptor mRNA in the suprachiasmatic nucleus (NSC) suggest that 5-HT_{1C} receptors may be important in controlling cyclic events (including cyclic changes in the reproductive neuroendocrine axis).

Interestingly, all areas containing serotonin-producing cells (i.e. raphe reticular formation and even the dorsomedial hypothalamic nuclei) express very high levels of 5-HT_{1C} receptor mRNA. Aghajanian and co-workers [33] have shown that activation of the 5-HT_{1A} receptor on the soma of raphe nuclei inhibits firing of serotonergic neurons. Similarly, the 5-HT_{1C} receptor may modulate or autoregulate the activity of serotonergic neurons.

Serotonin and catecholamines have opposing effects in controlling sleep, hypothalamic functions and motor activity [1]. Interestingly, the 5-HT_{1C} receptor transcript is expressed in all catecholamine (CA) cell group areas except for the hypothalamic CA cells of the periventricular and arcuate nuclei. This receptor may mediate serotonin interactions with catecholaminergic cells.

Prior to the pharmacological characterization of serotonin receptor subtypes, Aghajanian [4] had described three receptors for 5-HT based on electrophysiological responses. Occupation of one of these receptors found on motoneurons and in the reticular formation increased the excitability of postsynaptic neurons to other neurotransmitters, particularly glutamate. This response is blocked by methysergide and cinanserin, classical serotonin antagonists. In addition, the 'motor syndrome' [36], a complex set of motor behaviors elicited by 5-HT agonists, appears to be mediated by excessive facilitation of 5-HT_1 receptors on motor neurons on the brainstem or spinal cord [4]. The hallmark of the 5-HT_{1C} receptor is its high affinity for both 5-HT and clasical 5-HT antagonists [22,23]. It is therefore particularly interesting that neurons in motor nuclei (oculomotorius, trigeminal, VII, vagus and hypoglossal, for example) express high levels of 5-HT_{1C} receptor mRNA. In addition, the subthalamic structures which are thought to control muscle activity [1] also express very high levels of this receptor mRNA. Taken toether, these data suggest that the 5-HT_{1C} receptor may play an im-

portant role in serotonin effects on motor activity and control.

Serotonin was first identified as a blood-borne agent which caused vasoconstriction via receptors on the smooth muscle of blood vessels [33]. However, the nucleus of the solitary tract which expresses significant levels of 5-HT_{1C} receptor mRNA comprises the autonomic component of blood pressure control and respiration. Thus, serotonin may affect blood pressure and respiration at the level of this nucleus in addition to affecting blood vessels directly.

We have mapped in detail the expression of a serotonin receptor mRNA in the rat CNS. The identification of many neurons expressing this receptor subtype suggests that the 5-HT_{1C} receptor may mediate a number of the central effects of serotonin in both normal and pathological conditions. With this information, it may now be possible to dissect the complex role of serotonin in the CNS and begin to understand the physiological significance of 5-HT receptor subtypes.

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