Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 6793–6797, September 1989
Neurobiology

5-HT1c receptor is a prominent serotonin receptor subtype in the central nervous system

(5-HT1c receptor/serotonin/*in situ* hybridization/central nervous system/neurotransmission)

SUSAN M. MOLINEAUX, THOMAS M. JESSELL, RICHARD AXEL, AND DAVID JULIUS

Howard Hughes Medical Institute, and Department of Biochemistry and Molecular Biophysics, College of Physicians and Surgeons, Columbia University, New York, NY 10032

Contributed by Richard Axel, June 1, 1989

ABSTRACT **Neurons in rat central nervous system (CNS) that express 5-HT1c receptor mRNA have been localized by *in situ* hybridization histochemistry. The 5-HT1c receptor is expressed in a wide variety of cortical and subcortical neurons including hippocampal pyramidal neurons, neurons within most of the central monoaminergic cell groups, neurons in thalamic sensory relay nuclei, and neurons involved in the central processing and regulation of nociceptive transmission. Therefore, the 5-HT1c receptor is a prominent but poorly characterized central subclass of serotonin (5-HT) receptor. The distribution of the 5-HT1c receptor within the CNS is considerably more widespread than that of the structurally and functionally related 5-HT2 receptor.**

The monoamine serotonin (5-hydroxytryptamine; 5-HT) functions as a neurotransmitter in the mammalian central and peripheral nervous systems (1). Within the brain, serotonergic neurons originate primarily in the raphe nuclei of the brainstem (2). The axons of these neurons project to most areas of the central nervous system (CNS) (3, 4), where they regulate a wide variety of sensory, motor, and cortical functions (5). The diverse actions of 5-HT are mediated by interactions with several distinct subclasses of 5-HT receptors. These multiple 5-HT receptors have been defined on the basis of their ligand-binding profile and include 5-HT1a, -b, -c, and -d; 5-HT2; and 5-HT3 subclasses (6–8). Moreover, individual 5-HT receptor subtypes interact with distinct guanine nucleotide binding proteins and trigger different second-messenger pathways. Activation of the 5-HT1a receptor results in an inhibition of adenylate cyclase, whereas activation of the 5-HT1c and 5-HT2 receptors activates phosphotidylinositol-specific phospholipase C, causing the release of the second messengers 1P3 and diacyl glycerol (9, 10).

The diverse physiologic actions of 5-HT may not only derive from the activation of different intracellular signaling pathways but also from the expression of the individual receptor subtypes in distinct regions of the brain. Selective pharmacologic ligands have been used to determine the regional distribution of 5-HT receptors in the mammalian CNS. 5-HT1a receptors are widely expressed in cortical and subcortical areas, whereas 5-HT2 receptors are detected at highest levels in the cerebral cortex (11, 12). The 5-HT1c subtype was first detected in choroid plexus epithelial cells (13–15) and is the only 5-HT receptor subtype expressed by these cells. However, the distribution of 5-HT1c receptors in other regions of the nervous system has been difficult because there are no selective ligands for this receptor subclass; most ligands that bind to 5-HT1c receptor also interact with 5-HT2 receptors.

Recently, cDNA clones encoding the 5-HT1a, 5-HT1c, and 5-HT2 receptors have been isolated and sequenced (16–18). The deduced amino acid sequences reveal that the 5-HT receptors share sequence and structural similarities with the family of guanine nucleotide-binding protein-coupled receptors thought to traverse the membrane seven times (19). The 5-HT1c and 5-HT2 receptors are highly conserved in their primary structure and define a new subfamily of receptors, whereas the 5-HT1a receptor is more closely related to the subfamily of adrenergic receptors. In this paper, we have used *in situ* hybridization techniques to identify the site of synthesis of 5-HT1c receptor mRNA within the rat CNS. We find that neurons that express 5-HT1c receptor mRNA are widely distributed in the brain and spinal cord, indicating that this receptor subtype is likely to play a prominent and previously unappreciated role in the central action of 5-HT. The distribution of labeled neurons implicates the 5-HT1c receptor, in particular in the processing and integration of sensory information and in the regulation of central monoaminergic systems.

MATERIALS AND METHODS

***In Situ* Hybridization.** Adult rats were perfused with 4% paraformaldehyde as described (20). The brain and spinal cord were removed, post-fixed for 2 hr, and cryo-protected in phosphate-buffered saline containing 20% sucrose at 4°C overnight. The tissue was frozen in M1 embedding matrix, and 12-μm cryostat sections were cut and thaw-mounted onto slides coated with gelatin and poly(L-lysine). Prehybridization buffer [50% formamide/0.9 M NaCl/10 mM Tris, pH 7.5/1 mM EDTA/250 μg of tRNA per ml/500 μg of salmon sperm DNA per ml/5× Denhardt's solution (1× = 0.02% polyvinylpyrrolidone/0.02% bovine serum albumin/0.02% Ficoll)] was applied to sections, and the slides were then covered with Parafilm and incubated for 1–3 hr at 50°C. Coverslips were then removed, and the slides were dehydrated through graded ethanols. Probe (1–4 × 10^6 cpm) was applied to the sections in a 150-μl volume of hybridization buffer [50% formamide/0.9 NaCl/10 mM Tris, pH 7.5/1 mM EDTA/50 μg of tRNA per ml/50 μg of yeast RNA per ml/10 μg of salmon sperm DNA per ml/10% dextran sulfate/50 μg of poly (A) per ml/0.08% bovine serum albumin/1× Denhardt's solution]. After hybridization at 50°C overnight, the slides were washed in 2× SSC (1× = 0.15 M NaCl/0.015 M sodium citrate, pH 7) at room temperature, followed by incubation in 20 μg of RNase per ml/0.5 M NaCl/10 mM Tris, pH 8, at 37°C for 45 min. High stringency washes were in 2× SSC/10 mM 2-mercaptoethanol at 50°C for 1 hr and 0.1× SSC/0.05% sodium pyrophosphate/10 mM 2-mercaptoethanol for 3 hr, after which the slides were cooled gradually to room temperature in the same solution overnight. After

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: 5-HT, serotonin (5-hydroxytryptamine); CNS, central nervous system.

dehydration through graded ethanols containing 0.3 M ammonium acetate, the slides were air-dried, dipped in Kodak NTB-2 emulsion, diluted 1:1 with 0.6 M ammonium acetate, and developed after 1–3 weeks.

Probe Synthesis. An RNA expression vector containing the 3-kilobase (kb) coding region of the 5-HT1c receptor cDNA was used to generate single-stranded RNA probes (17) in an *in vitro* transcription reaction containing 1 μg of linearized DNA template, 25 μM ^{35}S-substituted UTP (UTP [^{35}S]; 1000 Ci/mmol; 1 Ci = 37 GBq), 40 mM Tris (pH 7.5), 6 mM $MgCl_2$, 2 mM spermidine, 10 mM NaCl, 10 mM dithiothreitol, 0.5 mM ATP, 0.5 mM CTP, 0.5 mM GTP, 12 units of RNasin, and 10–30 units of either T3 polymerase or T7 polymerase to generate probes of opposite orientation. Reactions were performed at 37°C for 2 hr and unincorporated nucleotides were removed by two sequential ammonium acetate ethanol precipitations. The specific activity of the probes was 0.5–1 $\times 10^9$ cpm/μg of RNA.

RESULTS AND DISCUSSION

Specificity of Hybridization. The distribution of 5-HT1c receptor mRNA in the brain and spinal cord was determined by *in situ* hybridization. UTP[^{35}S]-labeled antisense RNA transcribed *in vitro* from an RNA expression vector containing a 5-HT1c receptor cDNA was used as probe (17). Hybridization with this antisense probe resulted in intense labeling of epithelial cells of the choroid plexus (Fig. 1*a*), whereas a sense of RNA transcribed from the same cDNA clone gave background levels of labeling (Fig. 1*b*). Treatment of tissue sections with RNase prior to hybridization completely abolished specific binding of the antisense RNA probe to cells in the choroid plexus and brain (not shown), providing evidence for the specificity of hybridization.

To exclude the possibility of cross-hybridization of the 5-HT probe with mRNAs encoding other structurally related receptors, particularly the 5-HT2 receptor, we used a 300-base-pair antisense RNA probe derived from the 3′ noncoding region of the 5-HT1c receptor cDNA. This probe is unlikely to hybridize with 5-HT2 receptor mRNA because the nucleotide sequences of the 5-HT1c and 5-HT2 receptors and of other members of this receptor superfamily diverge considerably at their 3′ ends (ref. 19 and unpublished work). The pattern of hybridization detected with antisense RNA probes derived from coding and noncoding regions was identical, indicating that under the present stringent hybridizing conditions, hybridization is selective for 5-HT1c receptor mRNA. We estimate from RNA blot analysis that the hybridization signal detected in the choroid plexus corresponds to about 40–50 mRNA copies per cell (17). Thus, the sensitivity of the present technique permits detection of 5–10 mRNA copies in neural cells, although in many neurons the intensity of hybridization approached that observed in the choroid plexus.

Distribution of 5-HT1c mRNA in Cortical Regions. Within the CNS, the most intense hybridization was detected in hippocampal pyramidal neurons (Fig. 1 *c* and *d*; Table 1). Pyramidal neurons in the ventral region of CA1, CA2, and CA3 were heavily labeled, whereas 5-HT1c receptor mRNA was not detected in the dorsal anterior region of the hippocampus or in the dentate gyrus (Figs. 2*a* and 3*a*). The abrupt transition in 5-HT1c receptor mRNA expression in pyramidal

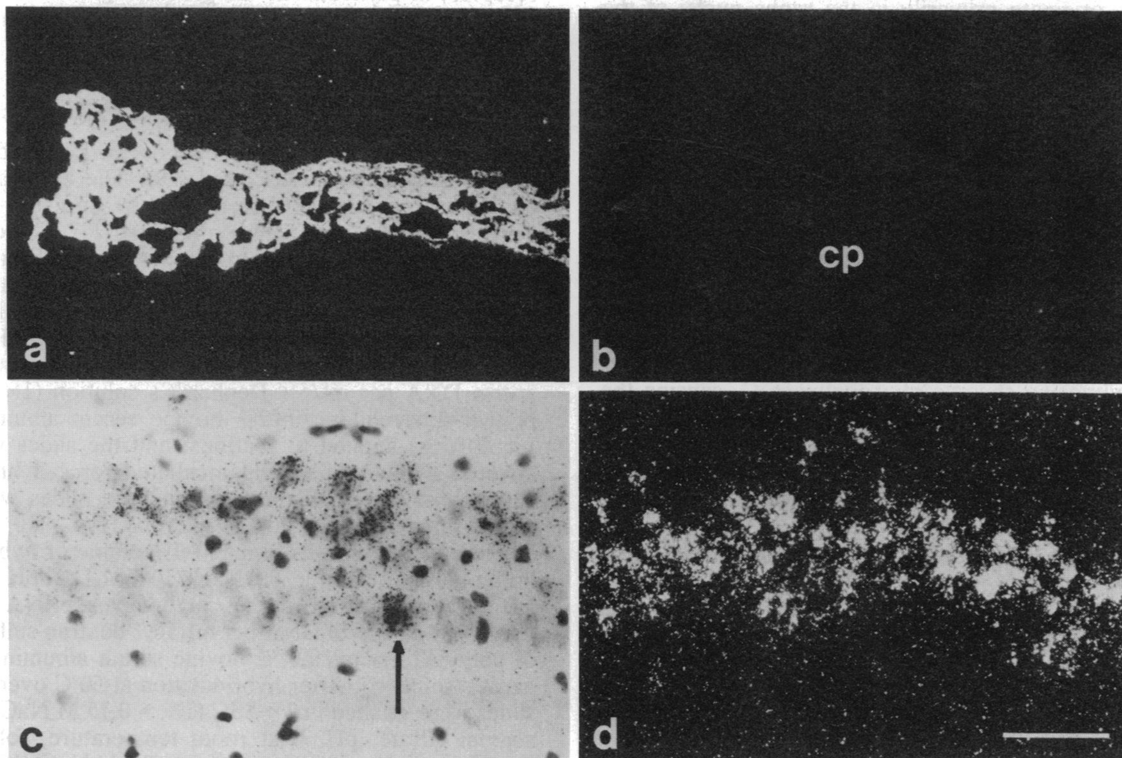

FIG. 1. Localization by *in situ* hybridization of 5-HT1c receptor mRNA in choroid plexus epithelial cells and hippocampal pyramidal neurons. (*a*) Intense labeling of choroid plexus epithelial cells is observed after hybridization with a 3-kb single-stranded antisense RNA probe for the 5-HT1c receptor mRNA. Adjacent cerebellar and midbrain tissue exhibits background levels of labeling. (*b*) Background grain density in choroid plexus (cp) is observed after hybridization of an adjacent section to that shown in *a* with sense-strand RNA probe derived from the same 5-HT1c receptor cDNA. (*c*) Labeling of a subset of hippocampal pyramidal neurons with 5-HT1c receptor RNA probe. Pyramidal neurons in the CA2/CA3 region were identified on the basis of their morphology in Nissl-stained sections. Unlabeled pyramidal neurons are clearly visible adjacent to a single heavily labeled pyramidal neuron (arrow). (*d*) Dark-field micrograph showing pyramidal neurons in the CA1/CA2 region with clusters of grains over virtually all pyramidal neurons in this region. Exposure time was 17 days. (Bar = 800 μm in *a* and *b*; 100 μm in *c* and *d*.)

Table 1. Distribution of neuronal cell bodies synthesizing 5-HT1c receptor mRNA

Neuronal cell body	Signal	Neuronal cell body	Signal
Olfactory system		Midbrain	
Anterior olfactory n.	++	Superior colliculus	+
Taenia tecta	++	Inferior colliculus	++
Primary olfactory cortex	+++	Central grey	++
Endopiriform n.	++	Substantia nigra, pars reticulata	++
Amygdala		Substantia nigra, pars compacta	+++
Anterior amygdaloid area	++	Ventral tegmental area	+
Central amygdaloid n.	++	Retrorubral field	++
Lateral amygdaloid n.	+++	Interpeduncular n.	+
Medial amygdaloid n.	++	Parabigeminal n.	++
Basal amygdaloid n.	++	Pontine oral reticular n.	++
Anterior cortical amygdaloid n.	++	Linear raphe n.	++
Amygdalohippocampal area	+	Median raphe n.	+
Thalamus		Dorsal raphe n.	++
Anteromedial thalamic n.	+	Dorsal tegmental n.	++
Laterodorsal thalamic n.	++	Midbrain reticular formation	++
Lateral posterior thalamic n.	++	Anterior pretectal area	+++
Central medial thalamic n.	++	Olivary pretectal area	++
Centrolateral thalamic n.	+	Posterior pretectal area	++
Intermediodorsal thalamic n.	+	Brainstem	
Paraventricular thalamic n., ant.	++	Locus ceruleus	++
Paraventricular thalamic n., post.	+	Central grey, pons	++
Posterior thalamic nuclear group	+	Pontine raphe n.	++
Paratenial thalamic n.	+	Raphe magnus	++
Lateral habenular n., caudal	+++	Raphe pallidus	+
Subthalamic n.	++++	Raphe pontis	+
Zona incerta	+	Raphe obscurus	+
Medial geniculate n.	++	Vestibular n.	+++
Hypothalamus		Prepositus hypoglossal n.	+
Anterior hypothalamic n.	++	n. of the solitary tract	++
Supraoptic hypothalamic n.	++	n. ambiguous	++
Suprachiasmatic n.	++	Parabranchial n.	++
Lateral hypothalamic area	++	Superior olive	++
Periventricular hypothalamic n.	+	Inferior olive	++++
Dorsomedial hypothalamic n.	+	Caudal pontine reticular n.	++
Supramammillary n.	+	Parvocellular reticular n.	+++
Lateral mammillary n.	+	Gigantocellular reticular n.	++++
Premammillary n., ventral	+	Reticular n. of the medulla	+++
Septohypothalamic n.	+	Paramedial reticular n.	+++
Preoptic area	+	Lateral reticular n.	+++
Hippocampus		Gracile n.	+
CA1 pyramidal layer, ventral	++++	Cuneate n.	+
CA2 pyramidal layer, dorsal	+++	Cerebellum	
CA2 pyramidal layer, ventral	++++	Deep cerebellar nuclei	+
CA3 pyramidal layer, ventral	++++	Spinal cord	
Subiculum, dorsal	+	Lamina V	+++
Subiculum, ventral	++++	Lamina VII	++

Grain densities were determined over neuronal cell bodies. Weak hybridization signals over neurons were designated +; moderate-intensity hybridization signals, ++; intense hybridization, +++; and intense hybridization over all neurons within a given region equivalent to that of choroid plexus, ++++. Nomenclature is from the atlas of Paxinos and Watson (21). n. = nucleus.

neurons can be observed most clearly in sections cut in a horizontal plane (Fig. 3*a*). The restricted expression of 5-HT1c receptor mRNA in the hippocampus suggests that 5-HT may exert different physiological functions within distinct hippocampal areas. 5-HT1a and 5-HT1b receptors have been identified physiologically on pyramidal neurons (22, 23), although there is no evidence that these two receptor subtypes are expressed on segregated subsets of pyramidal neurons.

The cerebral cortex contained few neurons that expressed 5-HT1c mRNA (Fig. 2 *a* and *b*). However, prominent hybridization was detected in the retrosplenial and anterior cingulate cortex (Figs. 2*b* and 3*b*). These two cortical regions have been implicated in spatial learning and memory (24). Therefore, the alterations in cognitive function that occur after administration of lysergic acid diethylamide and other related compounds (25) may be mediated in part by binding to these cortical 5-HT1c receptors. In other areas of the cerebral cortex, hybridization to layer 5 cells was detected in somatosensory and auditory cortex (Fig. 2*b*). The regional distribution of 5-HT2 receptor mRNA determined by RNA blot analysis indicates that the highest density of this receptor is found in the cerebral cortex, with 5- to 10-fold lower levels in other regions of the brain (26). These observations, together with the present findings, implicate both 5-HT1c and 5-HT2 receptors in the serotonergic regulation of cortical function.

Distribution of 5-HT1c mRNA in Subcortical Regions. In contrast to the 5-HT2 receptor, the 5-HT1c receptor is also expressed at high levels in many subcortical areas (see Table

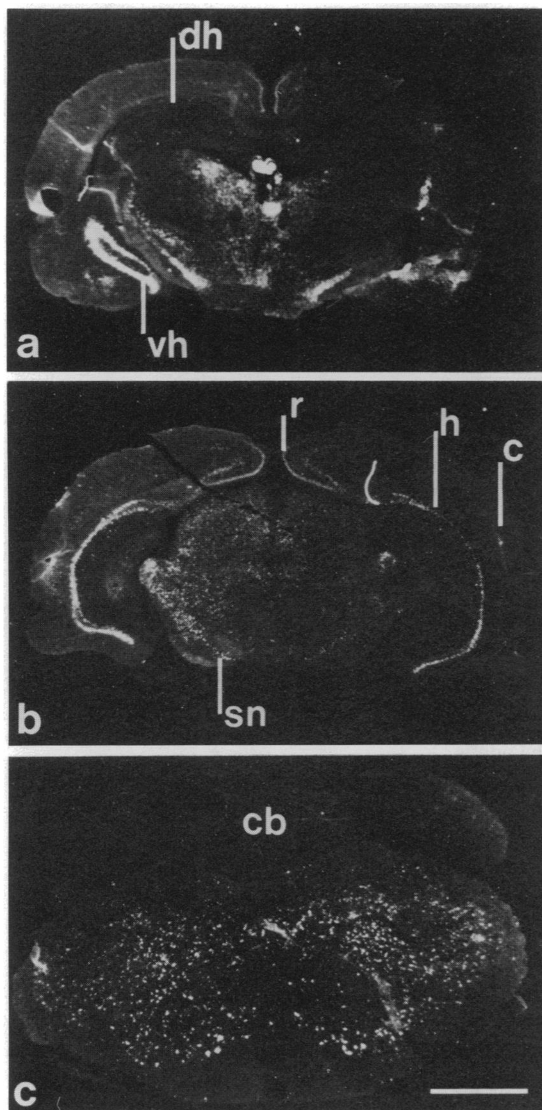

FIG. 2. Discrete localization of 5-HT1c mRNA in adult rat brain. (*a*) A coronal section through the diencephalon showing the distribution of 5-HT1c receptor mRNA. Intense labeling of pyramidal neurons is detected in ventral hippocampus (vh), whereas the dorsal hippocampus (dh) at the same rostral–caudal level is unlabeled. (*b*) A coronal section through the mesencephalon showing the distribution of 5-HT1c receptor mRNA in CA1 and CA2 hippocampal pyramidal neurons (h). Intensely labeled neurons are present in the substantia nigra (sn) and the central grey region. Some layer 5 cortical neurons (c) are also labeled. (*c*) A coronal section through the medulla showing grains over cells in the raphe obscurus and raphe pallidus and throughout the reticular core. The cerebellum (cb) is unlabeled. (Bar = 3 mm in *a*, 3.5 mm in *b*, and 1.8 mm in *c*.)

1). Neurons in a variety of thalamic and midbrain relay nuclei that convey sensory input to cortical structures exhibited strong hybridization (Fig. 2 *a* and *b*). Prominent labeling was observed in the medial geniculate nucleus (Fig. 3*d*) and the posterior thalamic subnuclei, which was consistent with a role for the 5-HT1c receptor in the serotonergic regulation of somatic and auditory sensory inputs. In addition, essentially all of the nuclei involved in the afferent and efferent connections of the limbic system expressed 5-HT1c receptor mRNA (Table 1).

There is also a striking expression of 5-HT1c receptor mRNA in monoaminergic cell groups (Table 1). A high density of neurons in the pars compacta of the substantia nigra expressed 5-HT1c receptor mRNA (Fig. 3*e*). Since most neurons in this region are dopaminergic (26), a substantial number of dopaminergic neurons must express 5-HT1c receptors. Previous studies have implicated serotonergic systems in the regulation of dopaminergic function (27), and our observations indicate that these actions may be mediated in part by the 5-HT1c receptor. Activation of the 5-HT1c receptor in a variety of cell types triggers inositolphospholipid hydrolysis and the mobilization of internal Ca^{2+} (10). Thus, one function of the 5-HT1c receptor may be to enhance dopamine release, either locally from dopaminergic dendrites within the substantia nigra or distally from the axon terminals of nigral neurons in the corpus striatum. Neurons in most other dopaminergic cell groups also exhibited 5-HT1c receptor mRNA (Table 1). Therefore, the 5-HT1c receptor-mediated regulation of dopaminergic function may contribute more generally to the interactions between these two monoaminergic systems.

The most prominent noradrenergic cell group, the locus ceruleus, also contained a high proportion of neurons expressing 5-HT1c receptor mRNA (Fig. 3*f*). Similarly, neurons within serotonergic cell groups in the brainstem expressed the 5-HT1c receptor. Labeled neurons were prominent in each of the raphe nuclei and in adjacent reticular nuclei (Fig. 2*c*; Table 1). The expression of 5-HT1c receptor mRNA in these nuclei suggests that this receptor subtype may function as an autoreceptor on serotonergic neurons (28). 5-HT1a and 5-HT1b receptors have previously been identified as autoreceptors on serotonergic neurons on the basis of electrophysiological studies (29). Therefore, the 5-HT1c receptor may function both in the autoregulation of serotonergic neurons and in the modulation of other central monoaminergic systems.

Finally, it is notable that several neuronal cell groups implicated in the processing of afferent sensory information and in the central regulation of nociception and pain express 5-HT1c receptor mRNA. The periaqueductal gray is the site of origin of excitatory neurons that project to brainstem nuclei and activate descending raphe-spinal neurons that terminate on ascending spinothalamic tract neurons in laminae of the dorsal horn (30). Neurons in each of these three cell groups express 5-HT1c mRNA. Therefore, the central actions of 5-HT in the endogenous regulation of pain transmission (31, 32) may be mediated in part by interactions with the 5-HT1c receptor subtype.

Neuronal Localization of 5-HT Receptor Subtypes. A more precise definition of the functional role of 5-HT1c receptor requires localization of the receptor on the neuronal surface. Although in rat there are no selective ligands for the 5-HT1c receptor (11), mesulergine is a relatively selective 5-HT1c receptor ligand in human brain (25). Several regions of the human brain appear to express high levels of 5-HT1c binding sites (33, 34), whereas our studies indicate that the corresponding areas in rat brain do not contain a high density of neurons expressing 5-HT1c receptor mRNA. In these regions, 5-HT1c receptors may be located on the terminals of afferent neurons that originate in other regions of the brain. The generation of antibodies to the rat brain 5-HT1c receptor will permit a more definitive localization of the 5-HT1c receptor. The identification of neurons that synthesize 5-HT1c receptors, taken together with the cellular location of the receptor, now permits physiological analysis of the role of this receptor in brain function.

Our findings reveal that the 5-HT1c receptor is a major subclass of 5-HT receptor within the CNS. Similar *in situ* hybridization analysis has not been performed with the cloned 5-HT2 receptor. However, RNA blot analysis indicates that this receptor is largely restricted to the cerebral cortex (unpublished work). Thus, the 5-HT1c and 5-HT2 receptor subtypes bind the same ligand and couple to identical signaling systems but exhibit distinct patterns of expression within the CNS. The existence of two distinct but

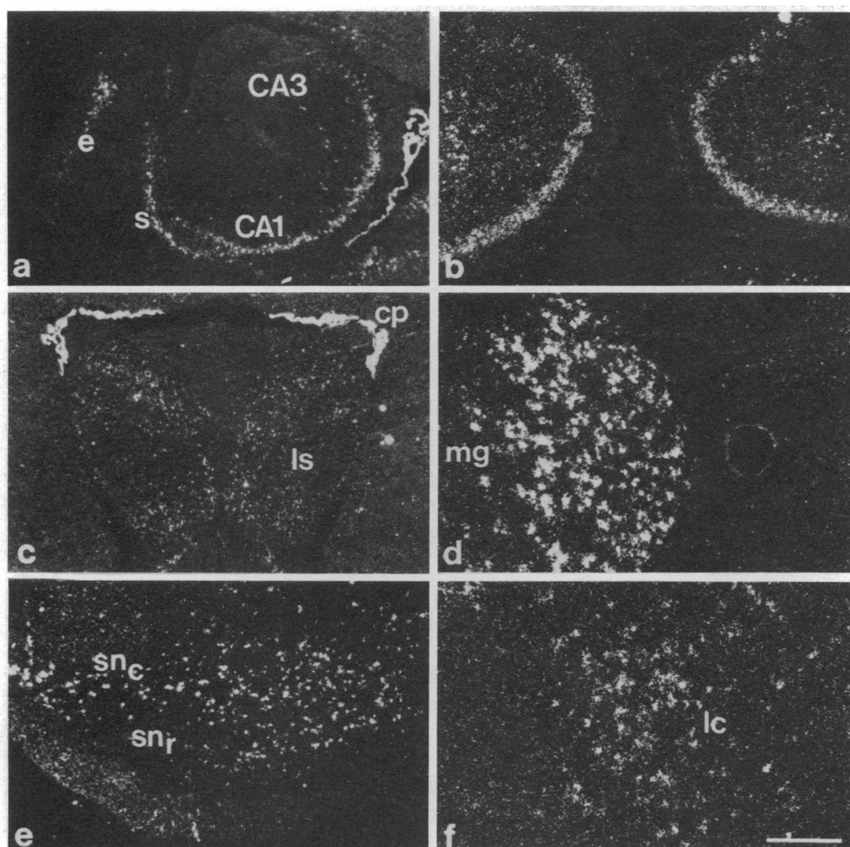

FIG. 3. Cortical and subcortical cell groups expressing high levels of 5-HT1c receptor mRNA. (*a*) A dark-field photomicrograph of a horizontal section through the ventral portion of the hippocampus showing the expression of 5-HT1c receptor mRNA in CA1, CA2, and in some CA3 pyramidal neurons. Neurons in the medial portion of CA3, as well as in CA4 and the dentate gyrus, are not labeled; however, entorhinal cortical neurons (e) and neurons in the subiculum (s) express 5-HT1c mRNA. (*b*) Coronal section revealing labeled neurons in the retrosplenial cortex. (*c*) A horizontal section showing neurons expressing 5-HT1c receptor mRNA in the lateral septal nuclei (ls) and choroid plexus (cp). (*d*) Localization of 5-HT1c receptor mRNA in the medial geniculate nucleus (mg). (*e*) Localization of neuronal cell bodies expressing 5-HT1c mRNA in the pars compacta (snc) and pars reticulata (snr) of the substantia nigra. (*f*) A horizontal section through the locus ceruleus (lc) showing the presence of neurons expressing 5-HT1c mRNA. Exposure time was 17 days. (Bar = 700 μm in *a*, 500 μm in *b*, 600 μm in *c*, 400 μm in *d*, 300 μm in *e*, and 125 μm in *f*.)

functionally analogous 5-HT receptor genes may provide a mechanism that permits the differential expression of these two receptors in different neuronal cell populations.

We thank Robert Fremeau and Mary Hynes for advice on *in situ* hybridization techniques, and Lillian Eoyang for technical assistance. R.A. and T.M.J. are investigators of the Howard Hughes Medical Institute, Columbia University.

1. Osborne, N. N. (1982) in *Biology of Serotonergic Transmission,* ed. Osborne, N. N. (Wiley, Chichester, U.K.), pp. 7–27.
2. Steinbusch, H. W. M. (1981) *Neuroscience* **6**, 557–618.
3. Azmitia, E. C. & Segal, M. (1978) *J. Comp. Neurol.* **179**, 641–667.
4. Parent, A., Descarries, L. & Beaudet, A. (1981) *Neuroscience* **6**, 115–138.
5. Vogt, M. (1982) in *Biology of Serotonergic Transmission,* ed. Osborne, N. N. (Wiley, Chichester, U.K.), pp. 299–315.
6. Peroutka, S. J. (1988) *Annu. Rev. Neurosci.* **11**, 45–60.
7. Peroutka, S. J. & Snyder, S. H. (1979) *Mol. Pharmacol.* **16**, 687–699.
8. Richardson, B. P., Engel, G., Donatsch, P. & Stadler, P. A. (1985) *Nature (London)* **316**, 126–131.
9. Kendall, D. A. & Nahorski, S. R. (1985) *J. Pharmacol. Exp. Ther.* **233**, 473–479.
10. Conn, P. J., Sanders-Bush, E., Hoffman, B. J. & Hartig, P. R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4086–4088.
11. Pazos, A. & Palacios, J. M. (1985) *Brain Res.* **346**, 205–230.
12. Pazos, A., Cortes, R. & Palacios, J. M. (1985) *Brain Res.* **346**, 231–249.
13. Pazos, A., Hoyer, D. & Palacios, J. M. (1984) *Eur. J. Pharmacol.* **106**, 539–546.
14. Yagaloff, K. A. & Hartig, P. R. (1985) *J. Neurosci.* **5**, 3178–3183.
15. Pazos, A., Hoyer, D. & Palacios, J. M. (1984) *Eur. J. Pharmacol.* **106**, 531–539.
16. Fargin, A., Raymond, J. R., Lohse, M. J., Kobilka, B. K., Caron, M. G. & Lefkowitz, R. J. (1988) *Nature (London)* **335**, 358–360.
17. Julius, D., MacDermott, A. B., Axel, R. & Jessell, T. M. (1988) *Science* **241**, 558–564.
18. Pritchett, D. B., Bach, A. W. J., Wozny, M., Taleb, O., Dal Toso, R., Shih, J. C. & Seeburg, P. H. (1988) *EMBO J.* **7**, 4135–4140.
19. Dohlman, H. G., Caron, M. G. & Lefkowitz, R. J. (1987) *Biochemistry* **26**, 2657–2664.
20. Fremeau, R. T., Lundblad, J. R., Pritchett, D. B., Wilcox, J. N. & Roberts, J. L. (1986) *Science* **234**, 1265–1269.
21. Paxinos, G. & Watson, C. (1982) *The Rat Brain in Stereotactic Coordinates* (Academic, Sydney).
22. Maura, G. & Raiteri, M. (1986) *Eur. J. Pharmacol.* **129**, 333–337.
23. Andrade, R., Malenka, R. C. & Nicoll, R. A. (1986) *Science* **234**, 1261–1265.
24. Sutherland, R. J., Whishaw, I. Q. & Kolb, B. (1988) *J. Neurosci.* **8**, 1863–1872.
25. Jacobs, B. L. (1984) in *Hallucinogens: Neurochemical, Behavioral, and Clinical Perspectives,* ed. Jacobs, B. L. (Raven, New York), pp. 183–202.
26. Moore, R. Y. & Bloom, F. E. (1978) *Annu. Rev. Neurosci.* **1**, 129–169.
27. Keller, J. J., Burkard, W. P., Pieri, L., Bonetti, E. P. & Da Prada, M. (1978) in *Advances in Biochemical Psychopharmacology,* eds. Roberts, P. J., Woodruff, G. N. & Iversen, L. L. (Raven, New York), Vol. 19, pp. 393–396.
28. Chesselet, M.-F. (1984) *Neuroscience* **12**, 347–375.
29. Middlemiss, D. N. (1986) *Eur. J. Pharmacol.* **120**, 51–56.
30. Dubner, R., Ruda, M. A., Miletic, M., Hoffert, M. J., Bennett, G. J. & Coffield, J. A. (1983) *Adv. Pain Res. Ther.* **6**, 151–166.
31. Basbaum, A. I., Mass, M. S. & Glazer, E. J. (1983) *Adv. Pain Res. Ther.* **5**, 323–339.
32. Yaksh, T. L. & Tyce, G. M. (1979) *Brain Res.* **171**, 176–181.
33. Hoyer, D., Pazos, A., Probst, A. & Palacios, J. M. (1986) *Brain Res.* **376**, 97–107.
34. Pazos, A., Hoyer, D., Dietl, M. M. & Palacios, J. M. (1988) in *Neuronal Serotonin,* eds. Osborne, N. N. & Hamon, M. (Wiley, Chichester, U.K.), pp. 507–543.