Brain Research, 561 (1991) 51–60

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ADONIS 000689939117019M

BRES 17019

Comparative anatomical distribution of 5-HT_{1A} receptor mRNA and 5-HT_{1A} binding in rat brain — a combined in situ hybridisation/in vitro receptor autoradiographic study

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(Accepted 14 May 1991)

Key words: $Serotonin_{1A}$ receptor; mRNA; In situ hyridization; Autoradiography; Neuroanatomy

The present study examined the comparative distribution of 5-HT_{1A} receptor mRNA and 5-HT_{1A} receptors in rat brain using a combination of in situ hybridisation histochemistry and in vitro receptor autoradiography. 5-HT_{1A} mRNA was visualised using a 910 bp cRNA probe synthesised from a *Bal*I–*Pvu*II fragment of the rat 5-HT_{1A} reetor gene, while 5-HT_{1A} receptors were labelled with the 5-HT_{1A}-selective ligand 8-OH-DPAT. In general terms, there was a complementary distribution of cells expressing 5-HT_{1A} receptor mRNA and 5-HT_{1A} receptor sites. High levels of both 5-HT_{1A} mRNA and 5-HT_{1A} receptors were evident in the hippocampal formation (CA1, CA3, dentate gyrus), entorhinal cortex, and raphe nuclei and lower levels in neocortex and thalamus. Although 5-HT_{1A} mRNA was not expressed in any regions which did not also exhibit 5-HT_{1A} receptors, within both the diagonal band and the medial septal nucleus mRNA levels were proportionately higher than 5-HT_{1A} receptor levels, possibly reflecting receptor transport or a heterogeneity in 5-HT_{1A} receptor turnover mechanisms. 5-HT_{1A} receptor mRNA and 5-HT_{1A} binding sites were undetectable in caudate/putamen and cerebellar regions. The present data indicate the synthesis of 5-HT_{1A} receptors both in raphe serotonergic cells and anatomically specific serotonergic projection areas, further supporting both a presynaptic autoregulatory and postsynaptic modulatory role for this receptor in serotonergic transmission.

INTRODUCTION

The midbrain raphe nuclei are the source of widespread serotonergic innervation throughout the brain[3] and serotonin (5-HT) is implicated in a diversity of physiological processes including thermoregulation, appetite, memory and sexual behavior[16]. Moreover, a large body of evidence supports a pathophysiological role for serotonergic dysfunction in anxiety and depressive disorders[5,6,9]. Based on ligand binding studies, 4 pharmacologically distinct populations of 5-HT receptors have been identified in the CNS; 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4[15]. Complex displacement of [^{3}H]5-HT binding by spiperone[19] first confirmed the heterogeneity of 5-HT_1 sites which have subsequently been classified into at least 4 receptor subtypes: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, and 5-HT_{1D}[12].

The development of selective molecules for the 5-HT_{1A} receptor has allowed identification of this site as both a putative inhibitory somato-dendritic autoreceptor on raphe serotonergic cells and as a postsynaptic receptor in selective serotonergic terminal fields[10]. In addition, anxiolytic[24] and antidepressant[20] actions of 5-HT_{1A}-selective compounds have generated much interest in this receptor in relation to both its role in general limbic function and possible involvement in the pathophysiology of depressive illness.

Recently, Albert and colleagues reported the cloning and functional characterisation of the rat 5-HT_{1A} receptor gene[1]. In common with other members of the G protein-coupled receptor family, the 5-HT_{1A} gene exhibits an intronless open reading frame encoding a protein with 7 putative transmembrane domains possessing both N-linked glycosylation sites in the N-terminal domain and potential phosphorylation sites in the third cytoplasmic loop. Here, we describe the anatomical distribution of cells in rat brain expressing 5-HT_{1A} mRNA using in situ hybridisation histochemistry (ISSH) and compare this distribution to that of radiolabelled 5-HT_{1A} receptor sites.

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MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (250–300 g) were sacrificed by decapitation, brains were rapidly removed and frozen in liquid isopentane (−42 °C). Subsequently, brains were sectioned on a cryostat maintained at −20 °C and thaw mounted onto polylysine-coated microscope slides. Sections were stored at −80 °C prior to ISSH and in vitro receptor autoradiography.

Riboprobe production

A *Bal*I–*Pvu*II fragment of the rat 5-HT_{1A} receptor gene, previously reported by Albert and colleagues[1], was ligated into *Hinc*II-cut pGEM blue (Promega). This fragment is composed of a 910 bp insert covering the sequence from the beginning of the second putative transmembrane domain to the middle of the extra-cytoplasmic domain found between transmembrane domains VI and VII, thus encompassing the entire sequence of the third cytoplasmic loop (the region of least homology for the G protein-coupled receptor family). Sense and antisense riboprobes were produced by linearising the plasmid with appropriate restriction endonucleases and transcribing using either SP6 or T7 promoter sites in the presence of [^{35}S]UTP (1000 Ci/mmol).

In situ Hybridisation

Sections were removed from storage at −80 °C and placed directly into 4% (v/v) formaldehyde at room temperature. After 60 min, slides were rinsed in isotonic phosphate buffered saline (2 × 5 min) and treated with proteinase K (1 μg/ml in 100 mM Tris/HCl, pH 8) for 10 min at 37 °C. Subsequently, sections underwent successive rinses in water (1 min), 0.1 M triethanolamine (pH 8, plus 0.25% acetic anhydride) for 10 min and 2 × SSC (0.3 mM NaCl, 0.03 mM sodium citrate, pH 7.2) for 5 min. Sections were then dehydrated through graded alcohols and air dried.

Post-fixed, sections were hybridised with 1.0 × 10^6 dpm [^{35}S]UTP-labelled 5-HT_{1A} riboprobes in hybridisation buffer containing 75% formamide, 10% dextran sulfate, 3× SSC, 50 mM sodium phosphate buffer (pH 7.4), 1× Denhardts solution, 0.1 mg/ml yeast tRNA, 0.1 mg/ml sheared salmon sperm DNA and 10 mM dithiothreitol in a total volume of 20 μl. Diluted probe was applied to sections on a glass coverslip which was sealed into place with rubber cement. These sections were then hybridised overnight at 55 °C.

Post-hybridisation, the rubber cement was removed and sections were washed in 2× SSC for 5 min. Slides were then treated with RNase A (200 μg/ml in 10 mM Tris/HCl, pH 8, containing 0.5 M NaCl) for 30 min at 37 °C. Subsequently, sections were washed in 2× SSC for 10 min, 1× SSC for 10 min, 0.5× SSC for 60 min at hybridisation temperature (55 °C), 0.5× SSC at room temperature for 10 min and then dehydrated in graded alcohols and air dried. For signal detection, sections were either placed on Kodak XAR-5 X-ray film and exposed for 3 days at room temperature or dipped in Kodak NTB-2 emulsion and stored dessicated in light tight boxes at 4 °C for 10 days.

In vitro receptor autoradiography

[^{3}H]8-OH-DPAT binding was performed according to previously published methods[18]. Briefly, slide-mounted tissue sections were preincubated in 0.17 M Tris/HCl, pH 7.6, containing 4 mM $CaCl_2$ and 0.1% ascorbic acid for 30 min at room temperature. Subsequently, sections were incubated with 2 nM [^{3}H]8-hydroxy-2-(*N*,*N*-di-*n*-propylamino) tetralin) for 60 min at room temperature. Postincubation, slides were washed in incubation buffer (2 × 5 min) at 4 °C and dried in a stream of cold air. Non-specific binding was determined in the presence of 2 μM 5-HT. Sections were apposed to ^{3}H-sensitive Hyperfilm and exposed at room temperature for 7 days. As 2 nM has previously been determined as a saturating concentration of 8-OH-DPAT[18], resulting receptor autoradiograms are a measure of B_{max} for 5-HT_{1A} receptors.

Microdensitometric analysis

Autoradiograms generated from ISHH and in vitro receptor autoradiography were analysed using an automated image analysis system (Dage camera, MAC II/IMAGE program). Anatomical regions of interest were interactively selected and mean optical density measurements determined from at least 3 coronal sections. For ISHH non-specific labelling was measured from an area of section in which labelling wasundetectable. Non-specific binding was determined in adjacent sections incubated with 2 μM 5-HT.

RESULTS

Specificity controls

Probe specificity was confirmed by both hybridising sections with labelled sense (mRNA) 5-HT_{1A} probe and by treating adjacent sections with RNase (200 mg/ml) prior to hybridisation with antisense (cRNA) 5-HT_{1A} probe. In both cases the subsequent signal was equivalent to background (Fig. 1). Non-specific binding of [^{3}H]8-OH-DPAT was determined in the presence of 1000-fold excess 5-HT and amounted to less than 5% of total binding.

Anatomical distribution

The comparative anatomical distribution of 5-HT_{1A} mRNA expression and 5-HT_{1A} receptor binding was determined in adjacent coronal and horizontal brain sections. Table I summarises the semi-quantitative analysis of both in situ hybridisation autoradiograms and ligand binding autoradiograms, while Figs. 2–6 visually illustrate this analysis.

Telencephalon

As can be seen from Figs. 2 and 4, the highest levels of 5-HT_{1A} mRNA expression in the telencephalon were observed in the hippocampal formation. However, within this structure the distribution of signal was heterogeneous; very high levels of labelling were evident in the pyramidal cell layer of both CA1 and CA3 subfields and only low levels in CA2 (Fig. 4). This distribution was closely matched by that of 5-HT_{1A} receptor binding (high in CA1 and CA3, low in CA2), however, within CA1 and CA3 subfields 5-HT_{1A} receptor sites were not confined to pyramidal cell layer but were also labelled across stratum oriens, radiatum and moleculare (Fig. 4B[1]). In a similar fashion, within the dentate gyrus, high levels of 5-HT_{1A} mRNA in the granule cell layer were matched by dense labelling of 5-HT_{1A} receptors across both the granule cell layer and molecular layer (Fig. 4).

Within the septum, both lateral and medal nuclei exhibited very high levels of 5-HT_{1A} mRNA expression (Fig. 3). Similarly, a significant number of intensely labelled cells were found within the vertical limb of the diagonal band (Figs. 3 and 5). However, with respect to 5-HT_{1A} receptor binding, it is of interest that both the

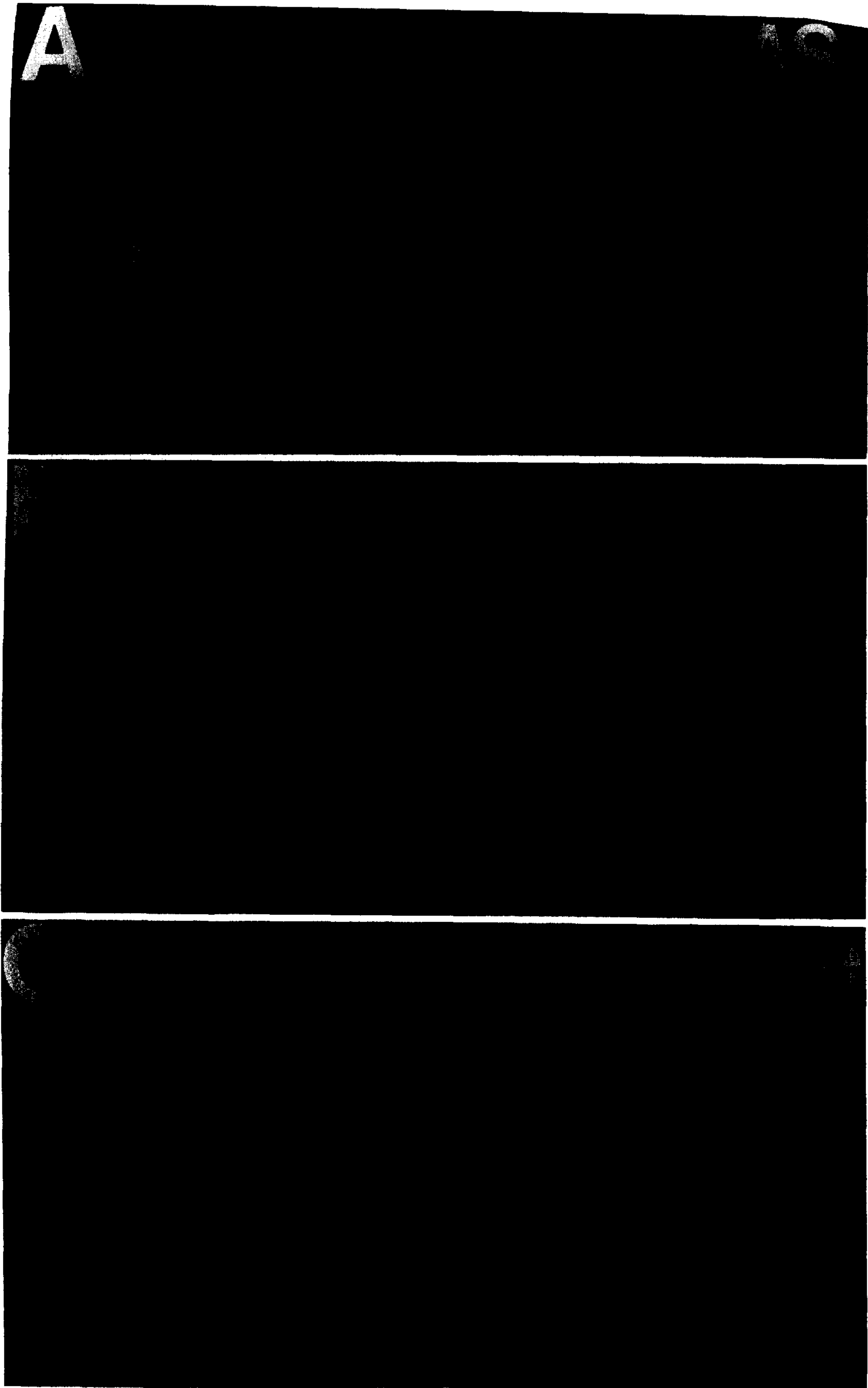

Fig. 1. Adjacent coronal sections incubated with antisense 5-HT_{1A} RNA probe (A), sense 5-HT_{1A} RNA probe (B), and antisense 5-HT_{1A} RNA probe after RNase pretreatment (C). Note the absence of specific labelling in B and C.

medial septal nucleus and the diagonal band could be characterised by proportionately lower levels of receptor sites than may be expected from 5-HT$_{1A}$ mRNA levels (Table I, Fig. 3). Although low levels of 5-HT$_{1A}$ mRNA were expressed in the bed nucleus of the stria terminalis, 5-HT$_{1A}$ message was undetectable within the caudate-putamen and nucleus accumbens (Fig. 3).

Within frontal, parietal and temporal regions of neocortex, cells expressing 5-HT$_{1A}$ mRNA were predominantly located in deeper cortical layers (IV–VI). In these areas, both in situ and receptor binding autoradiograms demostrated two distinct bands of complementary labelling (Fig. 3). In contrast, within the archicortical region of entorhinal cortex, intensely labelled clusters of 5-HT$_{1A}$ mRNA expressing cells were primarily located in a single superficial lamina (Fig. 4C^{I}), while 5-HT$_{1A}$ receptors were labelled across the entire cortical mantle (Fig. 4C^{I}). The density of both 5-HT$_{1A}$ mRNA and 5-HT$_{1A}$ receptor sites was relatively homogeneous across cingulate cortex.

Selective nuclei of the amygdaloid complex, cortical amygdaloid nucleus and basomedial amygdaloid nucleus, exhibited significant levels of 5-HT$_{1A}$ mRNA. This distribution was closely mathced by the distribution of 5-HT$_{1A}$ receptor binding (Fig. 4).

TABLE I

Semiquantitative evaluation of 5-HT$_{1A}$ receptor mRNA and 5-HT$_{1A}$ binding distribution in various brain regions

For both ISHH and in vitro receptor autoradiography optical densities for each anatomical region were determined as described in Materials and methods. Optical densities for each parameter are presented according to their respective percentile distributions: ++++ (>75%), very dense; +++(<75%, >50%), dense; ++(<50%, >10%), moderate; +(<10%), low, undetectable.

Anatomical region	*5-HT$_{1A}$ mRNA abundance*	*5-HT$_{1A}$ binding density*
Anterior olfactory nucleus	++	++
Amygdala; Basomedial nucleus	+++	+++
Cortical amygdaloid nucleus	+++	+++
Bed nucleus stria terminalis	+	++
Caudate nucleus	–	–
Cingulate cortex (superficial)	+++	+++
Cingulate cortex (deep)	+++	+++
Diagonal band	++++	++
Dorsal raphe nucleus	++++	++++
Entorhinal cortex (superficial)	++++	++++
Frontal cortex (superficial)	++	++
Frontal cortex (deep)	+++	+++
F/parietal cortex (superficial)	+	++
F/parietal cortex (deep)	++	+++
Hippocampal formation;		
CA1	++++	++++
CA2	+++	++
CA3	++++	++++
Dentate gyrus (granule/molecular)	++++	++++
Hypothalamus;		
Anterior nucleus	++	+++
Ventromedial nucleus	++++	+++
Inferior colliculus	+	++
Interpeduncular nucleus	++++	+++
Lateral septal nucleus	+++	++++
Medial septal nucleus	++++	++
Medial geniculate nucleus	+	+
Nucleus accumbens	–	–
Raphe magnus nucleus	++	++
Superior colliculus (superficial layer)	++	++
Ventral lateral geniculate nucleus	++	++
Zona incerta	++	+

Diencephalon

In general terms, compared to the telencephalon, few regions of the diencephalon exhibited 5-HT$_{1A}$ mRNA expression or receptor binding. Within the thalamus, the zona incerta demonstrated moderate levels of both 5-HT$_{1A}$ mRNA and 5-HT$_{1A}$ binding (Fig. 4A^{I}), as did the ventral lateral geniculate nucleus. 5-HT$_{1A}$ mRNA was undetectable in other major thalamic nuclei.

In the hypothalamus, the ventromedial and anterior hypothalamic nuclei exhibited dense to moderate levels of 5-HT$_{1A}$ mRNA, respectively (Fig. 4A^{I}, B^{I}). A distribution mirrored by 5-HT$_{1A}$ receptor binding. With the exception of these nuclei, however, 5-HT$_{1A}$ mRNA expression within hypothalamic regions, including the paraventricular nucleus, was unremarkable.

Mesencephalon

Both the dorsal and median raphe nuclei of the midbrain displayed high levels of 5-HT$_{1A}$ mRNA. Within the dorsal raphe, the most intensely labelled cells were evident in the more lateral and dorsal aspects of the nucleus while signal in the median raphe nucleus was predominantly located along the midline (Fig. 6). Both nuclei demonstrated 5-HT$_{1A}$ receptor binding in proportion to the level of 5-HT$_{1A}$ mRNA expression (Fig. 4C^{I}). At this level, scattered cells within the inferior colliculus demonstrated low levels of 5-HT$_{1A}$ mRNA (Fig. 4C^{I}), while, more rostrally, the superior colliculus also exhibited low levels of labelling, predominantly in the superficial gray layer. Diffuse 5-HT$_{1A}$ binding was evident within both areas. Very high levels of 5-HT$_{1A}$ mRNA were also expressed in the interpeduncular nucleus, with the most densely labelled cells being in the lateral subnucleus. Again, this high level of 5-HT$_{1A}$ mRNA expression was matched by high levels of 5-HT$_{1A}$ receptor sites.

Aside from some scattered cells in the lateral lemniscal nucleus, 5-HT$_{1A}$ mRNA was undetectable in other mesencephalic structures including the substantia nigra and ventral tegmental area.

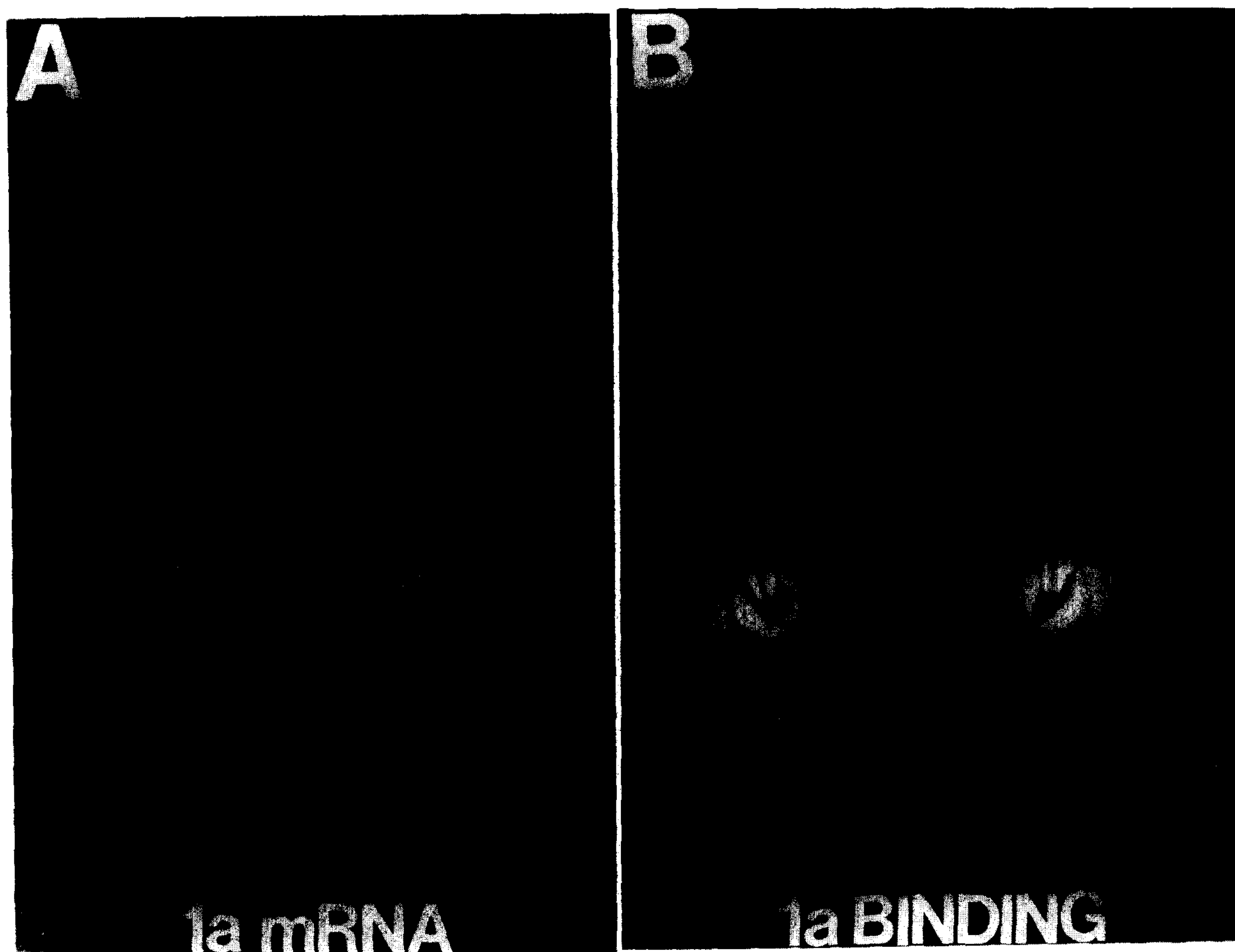

Fig. 2. Representative horizontal brain sections illustrating the general complementary distribution of 5-HT$_{1A}$ mRNA and 5-HT$_{1A}$ receptor sites.

Cerebellum, pons and medulla

Both 5-HT$_{1A}$ receptor mRNA and 5-HT$_{1A}$ receptor binding were undetectable in cerebellar cortex. Aside from a moderate level of expression in the raphe magnus nucleus, 5-HT$_{1A}$ receptor mRNA abundance was relatively unremarkable in pons and medulla.

DISCUSSION

In the present study, the combined use of ISHH and in vitro receptor autoradiography has allowed precise anatomical localisation and comparison of cells expressing 5-HT$_{1A}$ mRNA and 5-HT$_{1A}$ receptor sites. The specificity of the hybridisation signal was confirmed by both the absence of specific labelling in sections incubated with sense 5-HT$_{1A}$ probe and the abolition of specific signal after pretreatment of tissue sections with RNase. In addition, the heterogeneous distribution of 5-HT$_{1A}$ mRNA within the brain and the absence of specific signal in anatomical regions of high cell density, such as cerebellum, argues in favour of probe specificity. It is of interest that Albert et al.[1] have previously reported the existence of 3 distinct mRNA species for the rat 5-HT$_{1A}$ receptor as other G protein-coupled receptors (including the human 5-HT$_{1A}$ receptor) have been shown to express only one form of mRNA. The functional significance of this phenomenon is, at present, unknown. However, the rat 5-HT$_{1A}$ receptor gene is intronless and it is likely that the 3 mRNA species result from different transcriptional start sites or polyadenylation sites. In the present ISHH study we have employed specific cRNA probes which recognise all 3 mRNA species.

In general terms, the results of the present study indicate a broad complementary distribution of cells expressing 5-HT$_{1A}$ mRNA and 5-HT$_{1A}$ receptor sites. High levels of mRNA were found in hippocampus, dentate

gyrus, septal nuclei, entorhinal cortex and raphe nuclei. In agreement with previous autoradiographic studies[10,18], these areas also exhibited moderate to high densities of 5-HT_{1A} receptor sites. Lower levels of both mRNA expression and 5-HT_{1A} binding were evident in neocortex, hypothalamus and thalamus. However, although there was a macroscopic concordance of mRNA and receptor sites throughout the brain, subtle differences were evident within specific anatomical regions. Within hippocampus, 5-HT_{1A} mRNA was exclusively located in the pyramidal cell layer of CA1 and CA3 subfields, while 5-HT_{1A} receptors were found in high density across stratum oriens, radiatum and moleculare in these subfield (Fig. 4). Such a differential distribution can, however, be rationalised in cytoarchitectural terms. Within these fields, pyramidal cells are characterized by possessing two main groups of dendrites; the apical, extending through the radiatum layer to the stratum lacanosum moleculare and the basal, branching into the stratum oriens[4]. Thus it would seem likely that 5-HT_{1A} receptors

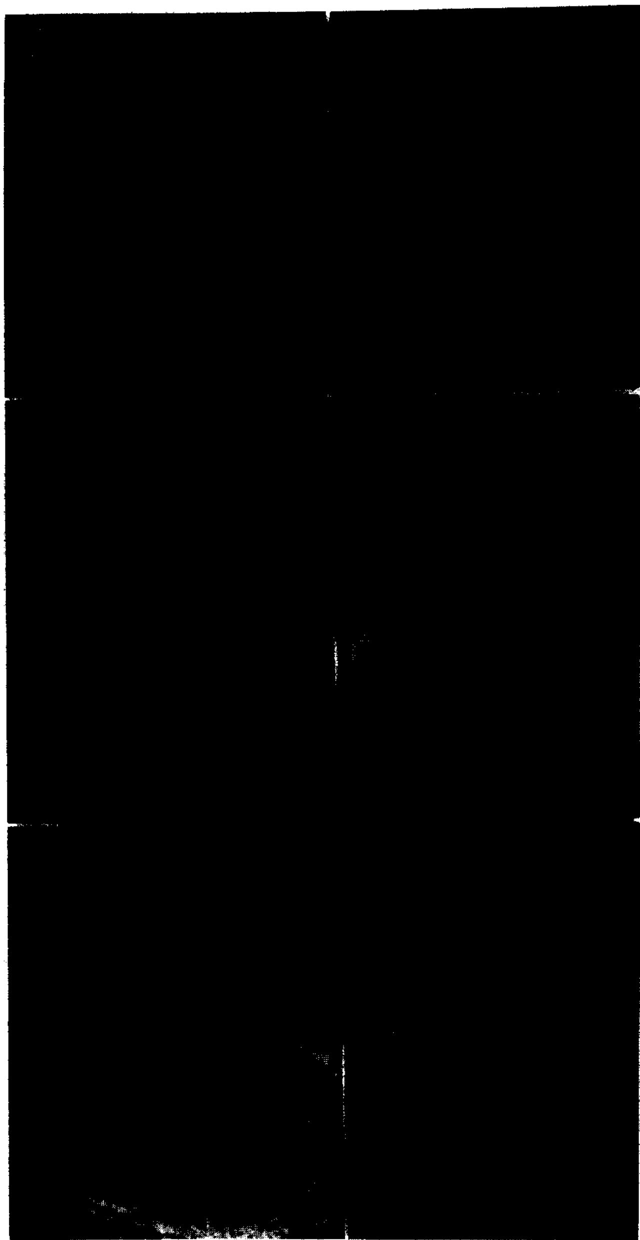

Fig. 3. Rostro-caudal (A–C) distribution of 5-HT_{1A} mRNA (left hemisphere) and 5-HT_{1A} receptors (right hemisphere) in representative coronal brain sections. Fcx, frontal cortex; AON, anterior olfactory nucleus; LS, lateral septal nucleus; MS, medial septal nucleus; VDB, vertical diagonal band. All images were generated from X-ray film.

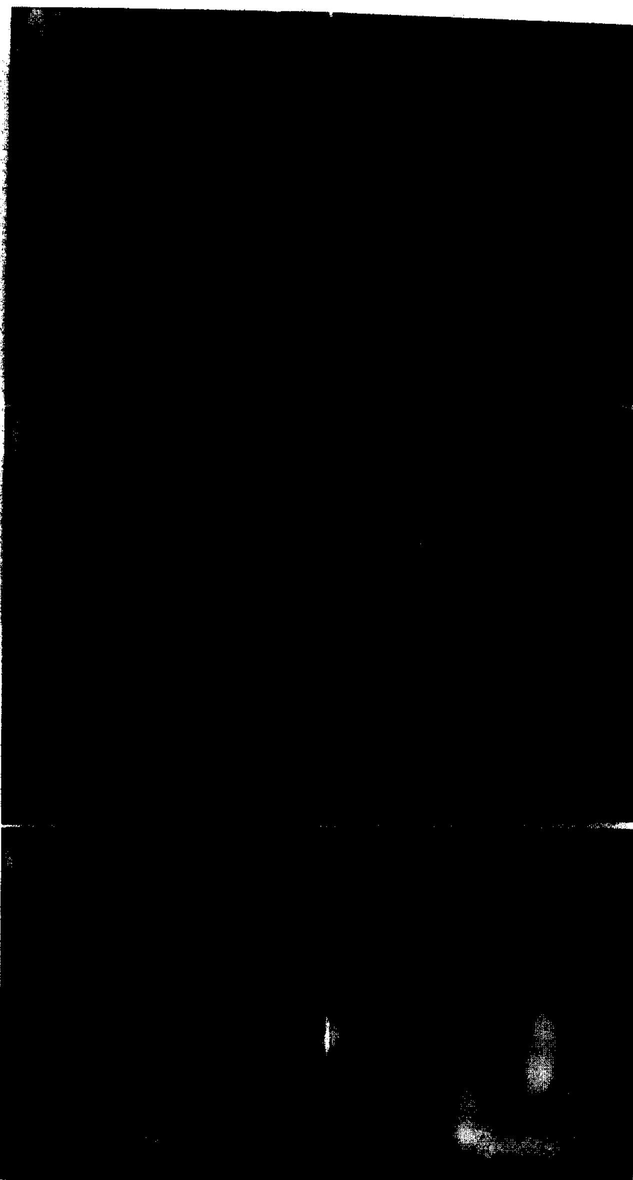

Fig. 4. Rostro-caudal (A^I–C^I) distribution of 5-HT_{1A} mRNA (left hemisphere) and 5-HT_{1A} receptors (right hemisphere) in representative coronal sections. CA1, field CA1 (Ammons horn); CA3, field CA3 (Ammons horn); DG, dentate gyrus; ZI, zona incerta; AH, anterior hypothalamic nucleus; BA, basolateral amygdaloid nucleus; Pir, piriform cortex; IC, inferior colliculus; DR, dorsal raphe nucleus; MR, median raphe nucleus; EnCx, entorhinal cortex. All images were generated from X-ray film.

Fig. 5. Dark-field photomicrographs of cells hybridised with [^{35}S]cRNA 5-HT_{1A} probe in (A) lateral septal nucleus (LS) and (B) vertical diagonal band (VDB). Bar = 100 μm.

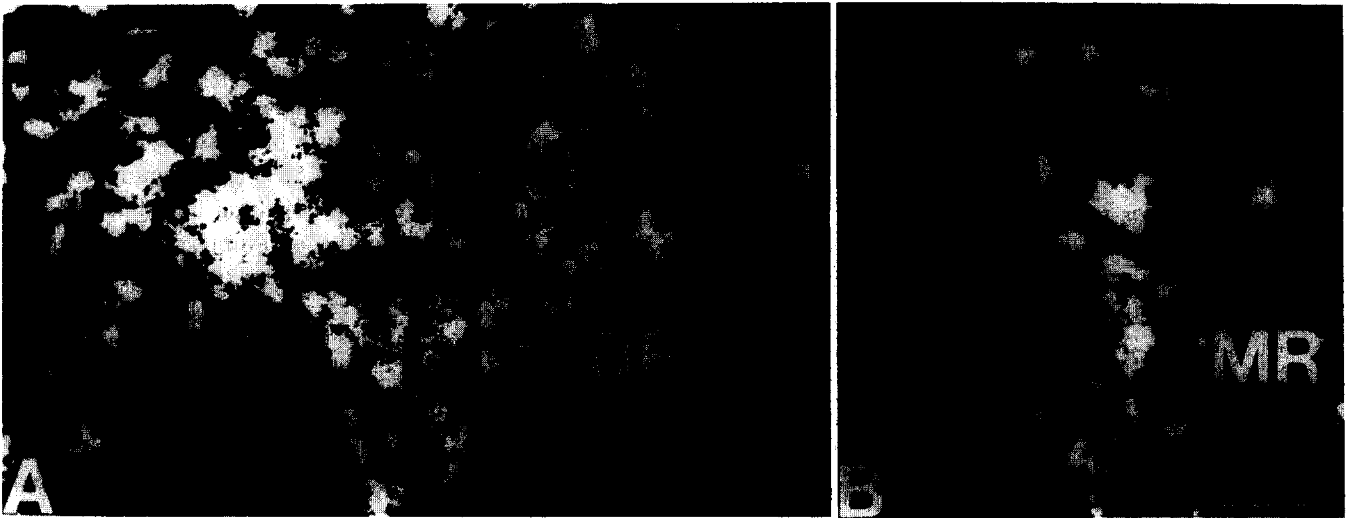

Fig. 6. Dark-field photomicrographs of cells hybridised with [^{35}S]cRNA 5-HT_{1A} probe in (A) dorsal raphe nucleus (DR) and (B) median raphe nucleus (MR). In A, note the high density of silver grains within cells in the more dorso-lateral aspect of the nucleus. In B, cells are hybridised along the midline. Bar = 60 μm. Note the high level of the anatomical resolution obtained in raphe nuclei from emulsion dipped sections in comparison to that obtained from X-ray generated images (Fig. 4).

synthesised in hippocampal pyramidal cell bodies are subsequently transported along both apical and basal dendrites prior to insertion into the neuronal membrane. In a similar fashion, 5-HT_{1A} receptors transcribed within the granule cell layer of the dentate gyrus appear to be transported to granule cell denritic fields in the molecular layer prior to insertion. While it may be feasible that a proportion of 5-HT_{1A} receptors could be located on afferent fibers to the hippocampus, both lesion studies[11] and electrophysiological data[12] indicate that hippocampal 5-HT_{1A} sites mediate postsynaptic responses to serotonergic input. The present data confirm the intrahippocampal synthesis of 5-HT_{1A} receptors, supporting a postsynaptic localisation.

In contrast to the hippocampus, within midbrain raphe nuclei 5-HT_{1A} receptors appear to be presynaptically located. Aghajanian and colleagues have shown that iontophoretic application of 5-HT_{1A} agonists onto raphe nuclei results in reduced firing of serotonergic neurons[21], suggesting an autoregulatory function for 5-HT_{1A} sites in this region. In keeping with these findings, the presence of 5-HT_{1A} mRNA and 5-HT_{1A} receptors in raphe nuclei strongly suggests a local synthesis and somato-dendritic localisation. Indeed, in agreement with the cytoarchitecture of the DR nucleus[23], higher levels of both 5-HT_{1A} mRNA expression and 5-HT_{1A} binding were evident in the lateral and dorsal aspects of the nucleus where the larger serotonin-producing cells are located. Interestingly, 5-HT_{1A} receptor mRNA has also been identified within raphe neurons[14] indicating that 5-HT_{1A} receptors may also subserve an autoregulatory function on serotonergic neurons. In the present study neuronal signal was identified by thionin staining. However, it is feasible to differentiate glial from neuronal signal by combining glial-specific immunocytochemical markers with in situ hybridisation. Such double labelling techniques could, however, produce false negative results where low abundance mRNA species are concerned due to the quenching of in situ signal by the immunolabelling.

5-HT_{1A} receptor mRNA was not found in any anatomical regions which did not exhibit 5-HT_{1A} receptor binding. However, the level of 5-HT_{1A} mRNA expression within any given anatomical region was not always directly related to the density of 5-HT_{1A} receptors in that region. This was the case in both the medial septal nucleus and the vertical diagonal band where, in comparison to other areas examined, mRNA levels were proportionately higher than receptor binding densities. Such a discrepancy may be explained in technical terms as ISHH using ^{35}S-labelled probes affords increased sensitivity as compared to ^{3}H-radioligands. It is also true that ^{3}H-radioligands are susceptible to tissue quenching artifacts which may compromise optical density measurements. However, given the anatomical specificity, it is possible that this disproportionality may reflect a specific neurobiological process, perhaps related to transport of the receptor protein to sites distant from the cell body transcribing the receptor mRNA or possibly related to 5-HT_{1A} receptor turnover mechanisms in these particular nuclei. The fact that both these areas are characterised by high densities of cholinergic cells adds some credibility to the possibility that 5-HT_{1A} receptors may be differentially regulated within different cell populations.

Serotonin is implicated in mood, behaviour and memory effects[6,16], central functions related to the limbic system. It is evident from the present study that the highest levels of 5-HT_{1A} mRNA expression are found in limbic structures; hippocampus, septum, entorhinal cortex, suggesting an important role for these sites in limbic circuits. In this respect, the anxiolytic and antidepressant effects of 5-HT_{1A}-selective compounds[20,24] indicates that 5-HT_{1A} receptor dysfunction may contribute to 5-HT_{1A}-associated affective disorders. Such a scenario is further suported by the ability of both tricyclic antidepressants and 5-HT uptake inhibitors to alter 5-HT_{1A} receptor numbers[25]. The ability of these drugs to alter 5-HT_{1A} mRNA expression remains to be investigated.

Serotonin is also known to influence a number of hypothalamic associated functions such as sleep, thermoregulation and neuroendocrine function. In this respect, multiple lines of evidence indicate that serotonin exerts a modulatory effect on the hypothalamic–pituitary–adrenal (HPA) system[2,7]. In particular, activation of 5-HT_{1A} receptors has been shown to induce corticotropin (ACTH) and corticosteroid release in both rodents[8] and human subjects[17], an effect antagonised by (–)-pindolol[8], a stereoselective $5\text{-HT}_{1A/1B}$ blocker. Although it is apparent that 5-HT_{1A} receptor activation regulates HPA function, the mechanism of action is unclear. One obvious possibility would be 5-HT_{1A} receptor mediated modulation of corticotropin-releasing hormone (CRH)-synthesizing neurons in the paraventricular nucleus of the hypothalamus, which, via the action of CRH, would result in increased ACTH and corticosteroid release. However, the lack of 5-HT_{1A} mRNA in the PVN in the present study argues against 5-HT_{1A} receptor synthesis in CRH neurons, suggesting that the site of action is not within the PVN. This raises the possibility that the modulatory effects of 5-HT_{1A} agonists are circuit mediated, possibly acting on other hypothalamic nuclei which subsequently project to PVN (anterior hypothalamic or ventromedial nuclei)[22] or on extra-hypothalamic structures. A hippocampal–bed nucleus–PVN circuit is stronlgy im-

plicated in negative feedback control of corticosteroid release[13] and could, theoretically, represent the mechanism of action for 5-HT_{1A} receptor mediated HPA modulation as both 5-HT_{1A} receptor mRNA and 5-HT_{1A} receptors were evident in the hippocampus and bed nucleus.

The present results using ISHH are generally consistent with Northern blot analysis of 5-HT_{1A} receptor mRNA in rat brain showing the highest levels of mRNA in hippocampus and septum and lowest in hypothalamus and basal ganglia[1]. However, the added sensitivity and anatomical resolution of ISHH has allowed identification of cells expressing 5-HT_{1A} mRNA in areas undetectable by Northern analysis, such as raphe nuclei and neocortex, and within cell groups in any anatomical region (e.g. pyramidal cells of CA1, CA3). In view of the importance of structure/function relationships in the brain, the ability to precisely localise cells expressing a given receptor type allows more insight into the putative function of the receptor within CNS circuits. Moreover, in combination with in vitro receptor autoradiography, ISHH provides valuable information not only in relation to possible pre- or post-synaptic localisation of receptors but also with respect to mechanisms of receptor synthesis and transport.

In conclusion, we have found a generally good concordance of cells expressing 5-HT_{1A} mRNA and the distribution of 5-HT_{1A} receptor binding. The high levels of 5-HT_{1A} mRNA expression within limbic structures and midbrain raphe nuclei suggests that this receptor plays an important role in mediating both serotonergic modulation of limbic circuits and general serotonergic tone. Future studies of 5-HT_{1A} receptor regulation may further our understanding of the importance of 5-HT receptor subtype specificity in mediating the neurophysiological actions of serotonin.

Acknowledgements. We thank Paul Albert and Olivier Civelli for sypplying the rat 5-HT_{1A} cDNA. These studies were supported by a Fogerty Fellowship to D.T.C. (No. 1 F05 TWO4452-01) and grants from NIMH (1 PO1 MH42251) and The Lucille P. Markey Charitable Trust (88-46) to S.J.W.

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