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[^{3}H]Tryptamine Autoradiography in Rat Brain and Choroid Plexus Reveals Two Distinct Sites[1]

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ABSTRACT

In vitro autoradiographic techniques were used to examine the distribution of [^{3}H]tryptamine binding sites in rat brain. The gross distribution and pharmacological characteristics of binding to brain sections resembled those seen in homogenate studies. Binding sites were found throughout the brain, with a preponderance of sites in the forebrain and limbic structures; highest levels were seen in the choroid plexus and the interpeduncular nucleus. Other regions exhibiting high levels of [^{3}H]tryptamine binding include the cortex (especially lamina I), caudate putamen, hippocampus, anterior olfactory nucleus, olfactory tubercle, nucleus accumbens, amygdala, superior colliculus (superficial gray layer), locus ceruleus, the nucleus of the solitary tract and the pineal body. Although there were similarities in this distribution to that for binding sites of [^{3}H]5-hydroxytryptamine ([^{3}H]serotonin), the overall patterns were distinct. The binding site for [^{3}H]tryptamine in the choroid plexus (termed T-2) was pharmacologically distinct from that in the rest of the brain (termed T-1); several compounds, including kynuramine, were potent inhibitors of [^{3}H]tryptamine binding at the brain site, but not at the choroid plexus site. [^{3}H]Serotonin also labels a site in the rat choroid plexus; this site was different from both [^{3}H]tryptamine sites. Knowledge of the distribution of tryptamine binding sites in the brain will aid in efforts to ascertain the function of these sites.

Several lines of evidence suggest that tryptamine may represent a neurotransmitter or neuromodulator. Tryptamine is distributed unevenly throughout the brain of rats and other species (Jones, 1982a; Boulton and Juorio, 1982), with higher concentrations in the caudate putamen, hypothalamus and hippocampus (Philips *et al.*, 1974). Although absolute concentrations of the amine are generally low (<1 ng/g; Philips *et al.*, 1974, 1980), its turnover is quite rapid in the brain, especially compared to 5-hydroxytryptamine (serotonin) (Philips and Boulton, 1979; Philips *et al.*, 1980; Durden and Philips, 1980). It can be formed locally by the decarboxylation of L-tryptophan (Saavedra and Axelrod, 1973; Warsh *et al.*, 1979), and appears to be localized to synaptosomal fractions (Boulton and Baker, 1975). When injected in the presence of monoamine oxidase inhibition, tryptamine produces several centrally mediated effects, including hyperalgesia (Larson, 1983), increased locomotor activity in rats (Foldes and Costa, 1975; Marsden and Curzon, 1978), hyperthermia and forepaw clonus in rabbits (Quock and Weick, 1978) and antagonism of head twitches induced by 5-hydroxytryptophan in mice (Jones, 1981). Although it is possible that some of the effects of tryptamine may be due to its interaction with serotonin receptors, Jones and colleagues demonstrated that tryptamine applied iontophoretically produced a postsynaptic depression of certain cortical neurons independent of serotonin effects (Jones and Boulton, 1980; Jones, 1982b,c).

The possibility that tryptamine may act as a neurotransmitter or neuromodulator (Jones, 1982a; Boulton and Jurio, 1982) received strong support with the discovery by Kellar and Cascio (1982) of a high-affinity binding site for [^{3}H]tryptamine in rat brain homogenates. Subsequent work by this group and others (Cascio and Kellar, 1982, 1983; Bruning and Rommelspacher, 1984; Wood *et al.*, 1984; Charlton et al., 1985) characterized further this site. There appears to be a single population of binding sites, with a K_D of ~2 to 3 nM, distributed unevenly throughout the brain (highest in hippocampus, striatum and cortex). The sites exhibit a unique pharmacology; few serotonergic compounds exhibit significant affinity. The compound with highest affinity was tryptamine itself; other compounds demonstrating significant affinity are the β-carbolines, including the harmaline family of drugs (Cascio and Kellar, 1981, 1982, 1983; Bruning and Rommelspacher, 1984; Wood *et al.*, 1984), phenylethylamine derivatives (Cascio and Kellar, 1983; Kellar and Cascio, 1986; Wood *et al.*, 1984) and kynuramine and related compounds (Charlton *et al.*, 1985). Inasmuch as representatives from each of these groups are found in brain tissue, the identity of the actual endogenous ligand(s) for the

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ABBREVIATIONS: THBC, tetrahydrobetacarboline; LSD, lysergic acid diethylamide; BOL, 2-bromo-LSD; B_{max}, maximum binding; CSF, cerebrospinal fluid.

[³H]tryptamine site remains uncertain. However, Cascio and Kellar (1986) demonstrated dramatic down-regulation of [³H] tryptamine binding in four brain regions after chronic pargyline administration, a treatment which has been shown to yield a 20-fold increase in mouse striatal tryptamine levels (Juorio, 1982).

Although the distribution of these sites was found to be uneven, the differences between regions was not great (Cascio and Kellar, 1983). *In vitro* receptor autoradiography, as developed by Kuhar and colleagues (Young and Kuhar, 1979; Unnerstall *et al.*, 1982), allows precise localization of neurotransmitter receptor binding sites, overcoming the lack of resolution inherent to homogenate binding studies. This communication describes the autoradiographical localization of binding sites for [³H]tryptamine in rat brain and other tissues, and demonstrates the presence of a unique binding site for [³H]tryptamine in choroid plexus. Preliminary findings were presented in abstract form (Perry *et al.*, 1982).

Materials and Methods

Materials. [³H]Tryptamine (26.5–41.5 Ci/mmol) was obtained from New England Nuclear (Boston, Ma.) [³H]Serotonin (13.1 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). Phenylethylamine, 5-methyltryptamine and THBC were generous gifts from Dr. Kenneth J. Kellar. Methysergide, fluoxetine, *d*- and *l*-amphetamine, LSD, spiperone, harmaline, phenylethylamine, BOL, ketanserin, mianserin, mesulergine, cinanserin, metergoline, cyproheptadine, probenecid, mazindol, propranolol, phenoxybenzamine and atropine were generous gifts from Dr. Solomon H. Snyder. All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). All drug concentrations were determined as free base.

Tissue preparation. Male Sprague-Dawley rats (150–250 g) were anesthetized with sodium pentobarbital and perfused intracardially with a 50:50 mixture of phosphate-buffered saline (pH 7.4) and 10% sucrose. Brains were then removed rapidly, frozen in dry ice powder and mounted onto brass stages using brain paste or Tissue Tek embedding medium (Miles Scientific, Naperville, IL). Sections (10 μ) were cut on a A.O. cryostat at −16°C and thaw-mounted onto gelatin-coated ("subbed") slides; sections were stored at −20°C until use.

Bovine brains were obtained fresh at a local slaughter house and transported to the laboratory on ice. Choroid plexus was dissected out, cut into pieces with scissors and homogenized in a glass-Teflon homogenizer in 0.32 M sucrose. The homogenate was centrifuged at low speed (500 × G for 10 min) to remove debris, and the supernatant was then centrifuged at 48,000 × *g* for 10 min. The resulting pellet was resuspended in buffer (50 mM Tris·HCl, pH 7.5 plus 5 mM ascorbic acid), incubated for 5 min at 37°C and then recentrifuged for 10 min at 48,000 × *g*. The resulting pellet was resuspended in binding buffer to give a tissue concentration of 50 to 200 mg (original wet weight/ml. Protein determinations were done using a BioRad (Richmond, CA) kit.

Homogenate binding. Binding was done in polypropylene tubes containing [³H]tryptamine (2.2–6.8 nM), competing compound and choroid plexus tissue (25–35 mg original weight) in a total of 1 ml of binding buffer (50 mM Tris·HCl, pH 7.5, 5 mM ascorbic acid and 10 μM pargyline). After a 60-min incubation on ice, mixtures were filtered with a Brandel (Gaithersburg, MD) filter unit over glass fiber filters (Schleicher and Scheull, Keene, NH) and rinsed with 3 × 4 ml of ice-cold binding buffer. [³H]Serotonin binding was done identically, except for the ligand concentration (13.4 nM), the addition of 4 mM $CaCl_2$ and the temperature (22°C). Filter-bound radioactivity was measured by liquid scintillation (Beckman 6800) in 7 ml of scintillation fluid (Safety-Solve; RPI, Inc., Mount Prospect, IL) after 60 min of agitation. Nonspecific binding was defined as that remaining in the presence of 10 μM concentration of the unlabeled ligand.

Autoradiography. Sections were thawed on a warming tray, preincubated for 15 min in 50 mM Tris.HCl, pH 7.5 at 4°C, then incubated at 4°C in a mixture of 50 mM Tris.HCl, pH 7.5, 5 mM ascorbic acid, 10 μM pargyline (to prevent destruction of ligand by monoamine oxidase) and 7% sucrose (to produce an iso-osmolar solution), containing [³H]tryptamine, with or without competing drugs. After incubation, sections were rinsed at 4°C in the same mixture without [³H] ligand. Initial experiments were performed to determine binding parameters; for these experiments, sagittal sections were wiped off the slide using a glass fiber filter, and the bound radioactivity was assayed by placing the filter into 7 ml of Safety-Solve, shaking for 60 min followed by liquid scintillation counting. Based on these experiments (see "Results"), subsequent experiments utilized an incubation time of 45 min, with two serial rinses of 10 min each. For these and other experiments, nonspecific binding was defined as that remaining in the presence of 10 *m*M tryptamine. [³H]Serotonin autoradiography was performed using identical conditins as for tryptamine; 10 μM serotonin was used to define the blank.

For saturation studies using wipes, increasing concentrations of [³H] tryptamine (0.1–10 nM) were added, either with or without 10 μM cold tryptamine to define nonspecific binding. For autoradiographic saturation studies, a "cold saturation" design was used in which the specific activity of the [³H]tryptamine was lowered progressively by increasing concentrations of cold tryptamine. For competition studies, incubations contained either [³H]tryptamine (1.0–7.6 nM) or [³H]serotonin (3.9–7.9 nM).

In autoradiographic experiments, after the incubation and rinse steps, slides were rapidly dried under a stream of cool dry air, and then apposed to tritium-sensitive film (LKB Ultrofilm, Rockville, MD) in the dark. After an exposure of from 40 to 90 days at 4°C, the film was developed (5 min with Kodak D-19 developer), fixed and dried. In some experiments, the coverslip method of Young and Kuhar (1979) was used instead of film. For these experiments, slides were glued to coverslips coated with Kodak NTB-4 emulsion in the dark; after similar exposure times the coverslips were then developed with Kodak D-19 and the sections stained with toluidine blue. Sections apposed to emulsion-coated coverslips were viewed for grain counting through a Leitz Orthoplan microscope with bright- and dark-field condensers; photomicrographs were obtained on Kodak Panatomic film with a Leitz Vario-Orthomat automatic exposure camera.

Analysis. Saturation and competition binding analysis were done on an IBM-PC using the programs EBDA (McPherson, 1983) and SCAFIT (LIGAND; Munson and Rodbard, 1980). In all cases examined a single-site model gave a significantly better fit than a two-site model. Some autoradiograms on LKB film were analyzed with a computerized video densitometry system by Loats Associates (Westminster, MD). Film response to tritium exposure was calibrated with the use of tritium standards (Microscales; Amersham); resulting optical densities were fit to a power curve by computer regression analysis to obtain a standard curve. These data were then analyzed as equilibrium binding data using the above mentioned program. Statistical comparisons of means were made using Student's *t* test; ranges throughout are expressed as ± S.E.

Results

Properties of [³H]tryptamine binding to rat brain sections. Initial experiments were performed to optimize binding conditions of [³H]tryptamine to tissue sections and ascertain the properties of this binding. These were done by counting radioactivity bound to sagittal sections wiped from slides. Optimal rinse time was determined by allowing binding to achieve equilibrium (60 min at 4°C), then transferring sections to fresh rinse buffer at 4°C for varying times. A rinse time of 20 min (two 10-min serial rinses) was found to yield the maximum ratio of specific to nonspecific binding (fig. 1A), with a total binding high enough to give a workable signal. When this rinse time was used in experiments to measure [³H]tryptamine association, equilibrium was reached by 30 min (fig. 1B); subse-

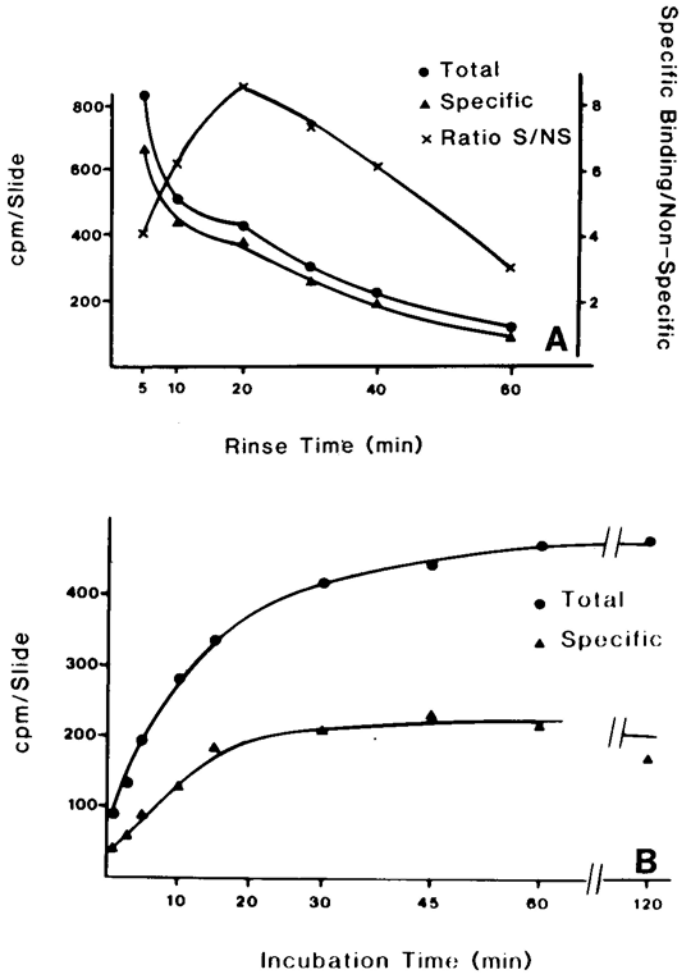

Fig. 1. Binding of [^{3}H]tryptamine to sagittal rat brain sections (wiped from slides and counted directly). Specific binding was obtained by subtracting the binding in the presence of 10 μM tryptamine (nonspecific binding) from the total. Each point is the mean of three sections; experiment was replicated twice. A, rinse times, after 60-min incubation. B, incubation times, with 2 × 10-min rinse.

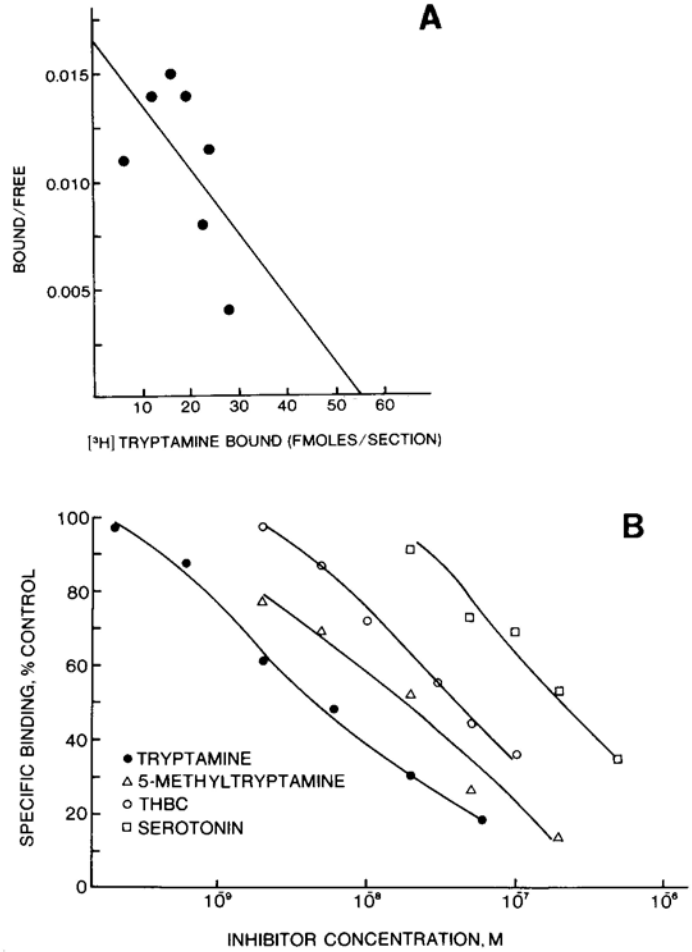

Fig. 2. Equilibrium analysis for [^{3}H]tryptamine binding to sagittal rat brain sections (wiped from slides and counted directly). A, scatchard-Rosenthal plot of specific binding from a typical saturation experiment; triplicate samples were incubated with 0.5 to 7.0 mM [^{3}H]tryptamine, either with (nonspecific binding) or without (total binding) 10 μM unlabeled tryptamine. B, binding competition curves for four compounds *vs.* 1.0 nM [^{3}H] tryptamine. Triplicate sections were incubated with different concentrations of unlabeled compound as indicated: tryptamine (●), 5-methyltryptamine (△); THBC (○); and serotonin (□).

quent experiments used a 45-min rinse time with 2 × 10 min serial rinses.

In order to determine whether the [^{3}H]tryptamine binding site behaved similarly with tissue sections and brain homogenates, the pharmacological specificity of this binding was determined by saturation and competition studies using wipes of sagittal sections. Saturation studies yielded a K_D value of 3.51 ± 0.60 nM (n = 6), with a B_{max} of 54.5 ± 13.8 fmol/section and a Hill coefficient n_H = 0.95 ± 0.04, suggesting a single class of binding sites. Figure 2A shows a Scatchard plot of one such saturation experiment. Competition experiments with several compounds (fig. 2B; table 1) were done to examine the pharmacological specificity of [^{3}H]tryptamine binding to brain sections. Tryptamine, 5-methyltryptamine and THBC all showed high affinity for the [^{3}H]tryptamine site, whereas serotonin showed only modest affinity (more than an order of magnitude lower than its affinity to 5-HT$_1$ serotonin receptors). LSD, which has high affinity for 5-HT$_1$ and 5-HT$_2$ sites, spiperone, which has high affinity for 5-HT$_2$ sites, and phentolamine, an *alpha* adrenergic antagonist, did not bind at all to the [^{3}H] tryptamine site (table 1). Thus, the [^{3}H]tryptamine binding site measured on rat brain sections appears to be identical to the site characterized previously in homogenate binding studies (Cascio and Kellar, 1982, 1983; Wood *et al.*, 1984; Bruning and Rommelspacher, 1984).

Distribution of binding sites. Coronal and sagittal sections from various regions were processed for autoradiography to determine distribution. Sites were widely distributed throughout the brain,[2] with a preponderance in rostral structures. Figure 3 represents autoradiographs of coronal and sagittal sections, at different brain levels. Nonspecific binding was only a small fraction of the total, as seen by comparing figures 3, K and L. The telencephalon contained many of the major brain regions high in [^{3}H]tryptamine sites. The cerebral cortex showed high levels of binding, especially in the frontal and entorhinal regions (fig. 3K). In all regions of the cortex, a significantly higher level of binding was seen in lamina I. In experiments with coverslips using grain counting for quantita-

[2] A detailed list of brain structures labeled, with relative binding densities, is on file with the American Society for Information Science National Auxiliary Publication Service, c/o Microfiche Publications, P.O. Box 3513, Grand Central Station, New York, NY 10017.

TABLE 1

Inhibition of specific [³H]tryptamine binding to sagittal rat brain sections and rat cortex homogenates

Values for sections are means of two experiments (points in triplicate). K_i values were calculated using a K_D for labeled ligand of 3.51 nM.

Compound	K_i, Section	K_i, Homogenates*
Tryptamine	4.9	2.4
5-Methyltryptamine	18.9	16
THBC	31.6	28
Serotonin	243	280
LSD	>10,000	>50,000
Spiperone	>10,000	>50,000
Phentolamine	>10,000	>50,000

* Cascio and Kellar, 1983.

tion, binding to lamina I of the frontal cortex was 135 ± 11% (n = 6) of that in laminae II to VI. In some autoradiographs, a slight decrease in labeling can be detected in lamina IV (figs. 3, C–F and 3K). The posterior cingulate cortex showed reduced levels of binding compared to other cortical regions (fig. 3F).

The gray matter of the caudate putamen (striatum) exhibited high levels of binding throughout (figs. 3, B–D, 3K and 5a). Other areas in the forebrain with high levels of binding include the anterior olfactory nucleus, the nucleus accumbens, the claustrum, the endopiriform nucleus, the lateral septum, the olfactory tubercle and Islands of Calleja. Moderately high levels were found in the lateral olfactory nucleus and the bed nucleus of the stria terminalis (figs. 3, B, C and K). Low to moderate levels were seen in the globus pallidus, the vertical limb of the diagonal band, the olfactory bulb, the medial septum and the ventral pallidum (figs. 3, A–C and 3K). White matter such as the olfactory tract, anterior commissure and corpus callosum exhibited only very low levels of specific binding.

Extremely dense binding of [³H]tryptamine to the choroid plexus was seen; this was equally intense in the 3rd, 4th and lateral ventricles (figs. 3, C–F, H, I, K, 5a and 6). In experiments using coverslips the grain density over the choroid plexus was 280 ± 13% (n = 6) of that in frontal cortex (lamina II–VI). Other telencephalic areas also showed appreciable levels of [³H] tryptamine binding. The amygdala was consistently high in binding, especially the cortical amygdaloid nucleus and the amygdalohippocampal area (figs. 3, D, E and K). The hippocampal structure was another major region for [³H]tryptamine sites (figs. 3, E, F and K). No major differences between the different fields were seen (*i.e.*, CA 1–4, dentate gyrus) except for a slight decrease in the subiculum. However, quite distinct layering was seen; the stratum oriens and stratum lacunosum-moleculare showed the densest binding, with reduced levels in stratum radiatum and still lower levels in the stratum pyramidale.

The diencephalon exhibited mostly moderate levels of [³H] tryptamine binding. Among thalamic nuclei, highest levels were seen in the paraventricular, paratenial, anteroventral, anteromedial, intermediodorsal, mediodorsal, laterodorsal, lateral posterior, dorsal lateral geniculate and reuniens thalamic nuclei (figs., 3, D–F, K and 5a). In the hypothalamus, the areas of greatest binding site density were the anterior and lateral hypothalmic areas, the arcuate nucleus, the periventricular nucleus and the medial and supramammillary nuclei (figs. 3, D–F, K and 5a). The medial habenular nucleus also exhibited moderately high levels of binding (fig. 3E).

Levels of binding in the mesencephalon varied greatly. The most striking finding was intense binding in the interpeduncular nucleus, especially the inner postsubnucleus (figs. 3, F and 5c). This represented the highest level of binding outside of the choroid plexus. Appreciable binding was also detected in the superficial gray (zonal) layer of the superior colliculus, and low to moderate levels in the pretectal nucleus, the periaqueductal gray, the substantia nigra, the nucleus of the optic tract and the dorsal raphe nucleus (figs. 3, F, G and K). Low levels are seen in the inferior colliculus, the median raphe nucleus, the medial and lateral geniculate nuclei and the post-thalamic nucleus.

Low to moderate levels of binding were seen in the granular and molecular layers of the cerebellum, as well as in the deep cerebellar nuclei (figs. 3, H–K). In the pons, binding was generally quite low; significant exceptions include high levels seen in the locus ceruleus and moderate levels in the pontine nuclei, the pontine central gray, and the parabrachial nuclei. Low to moderate levels were seen in the trapezoidal body and the ventral cochlear nucleus. The medulla was similarly low in [³H]tryptamine binding; notable exceptions include moderate levels seen in the gracile nucleus, the nucleus of the solitary tract, the area postrema, the medial vestibular nucleus, the dorsal cochlear nucleus, the inferior olive, the hypoglossal nucleus and the dorsal motor nucleus of the vagus (figs. 3, I and J). Low levels were seen in the parvocellular reticular nucleus, the external cuneate nucleus and the trigeminal and facial nerve nuclei. Both the dorsal and ventral horns of the spinal cord exhibited low to moderate levels of binding.

Other tissues also displayed [³H]tryptamine binding sites. Figure 4 shows the autoradiographical distribution in a sagittal section of a rat kidney. Dense labeling is apparent throughout the cortex; low levels were present in the medulla and none in the pelvis and ureter. High affinity specific binding for [³H] tryptamine in rat kidney homogenates was reported previously by Bruning and Rommelspacher (1984); the site they described showed a similar affinity and pharmacological profile to the site in rat cerebral cortex. Relatively high levels of [³H]tryptamine binding were also detected in the pineal body (fig. 5c).

Comparison to serotonin sites. The similar structures of tryptamine and serotonin (5-hydroxytryptamine) suggested the possibility that tryptamine sites may be related to serotonin receptors. Accordingly, the autoradiographical distribution of [³H] serotonin was compared to that of [³H]tryptamine; comparisons of the two ligand distributions at three brain levels are presented in figure 5. Several major areas of overlap are apparent, including the cortex, caudate putamen, choroid plexus, hippocampus, thalamus and the interpeduncular nucleus. However, several regions demonstrated distinctly different labeling patterns for the two ligands, including the cerebellum, globus pallidus and substantia nigra. The pineal body showed dense tryptamine labeling (fig. 5c) but almost no serotonin labeling (fig. 5f).

Densitometric analysis by regions. Results of saturation studies for tryptamine binding in six brain regions are shown in table 2; results were obtained using computerized densitometric analysis. The density of specific binding to the choroid plexus is almost three times the density of binding in the next highest region, the interpeduncular nucleus, and eight times the density in cortex and hippocampus. The interpeduncular nucleus has over three times the density of sites seen in the cortex or hippocampus. Interestingly, the corpus callosum, which like essentially all white matter in the brain appeared to have little or no binding, does demonstrate specific [³H]tryp-

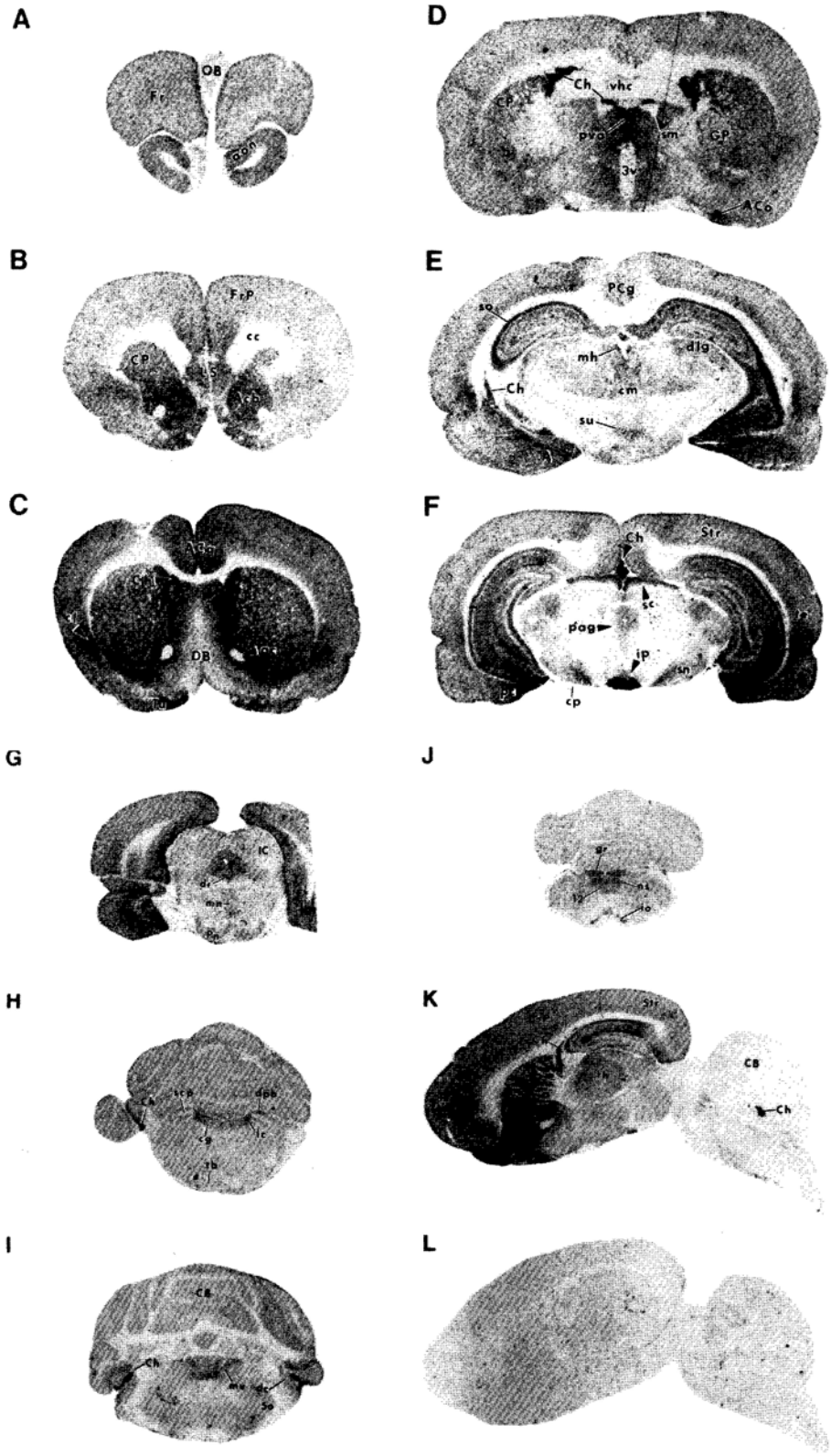

Fig. 3. Autoradiographic images of [^{3}H]tryptamine binding to various brain regions; conditions as in "Materials and Methods." All images represent total [^{3}H]tryptamine binding except L, which is a blank (a serial section to K, with 10 μM unlabeled tryptamine added to the incubation). The images correspond to the following images in the brain atlas of Paxinos and Watson (1982): A, 4 to 5; B, 10; C, 14; D, 17; e, 22 to 23; F, 26; G, 30; H, 34; I, 36 to 37; J, 42; and K and L, 50. Abbreviations: 3v, third ventricle; 5o, nucleus of the spinal tract of the trigeminal nerve, oral; 7, facial nucleus; 12, hypoglossal nucleus; A, amygdala; ac, anterior commissure; Acb, nucleus accumbens; ACo, anterior cortical amygdaloid nucleus; ACg, anterior cingulate cortex; aon, anterior olfactory nucleus; CB, cerebellum; cc, corpus callosum; cg, central gray pons; Ch, choroid plexus; cl, claustrum; cm, central medial thalamic nucleus; cp, cerebral peduncle; CP, caudate putamen; DB, diagonal band (vertical limb); dc, dorsal cochlear nucleus; dlg, dorsal lateral geniculate nucleus; dpb, dorsal parabrachial nucleus; dr, dorsal raphe nucleus; Fr, frontal cortex; FrP, frontoparietal cortex; GP, globus pallidus; gr, gracile nucleus; IC, inferior colliculus; io, inferior olive; ip, interpeduncular nucleus; lc, locus ceruleus; LS, lateral septum; mh, medial habenular nucleus; mn, median raphe nucleus; mv, medial vestibular nucleus; ns, nucleus of the solitary tract; OB, olfactory bulb; pc, posteromedial cortical amygdaloid nucleus; PCg, posterior cingulate cortex; Pn, pontine nuclei; sc, superior colliculus, superficial gray layer; Scp, superior cerebellar peduncle; sm, stria medullaris thalamus; sn, substantia nigra; so, stratum oriens, hippocampus; Str, striate cortex; sa, supramammilary hypothalamic nucleus; te, temporal cortex; th, thalamus; Tu, olfactory tubercle; tz, trapezoidal body.

tamine binding. The density is one-sixth that of the cortex or hippocampus, but the affinity constant suggests that these are the same class of sites seen elsewhere in the brain. Film autoradiography with tritium-labeled compounds is limited by unequal quenching in gray and white matter regions (Kuhar and Unnerstall, 1985); thus, caution is necessary when comparing regions such as the corpus callosum and cortex. Nevertheless densitometric analysis *did* reveal the presence of specific high-affinity binding in the corpus callosum which was not apparent on visual inspection. The slopes of the competition curves and the affinities were similar for gray and white matter regions.

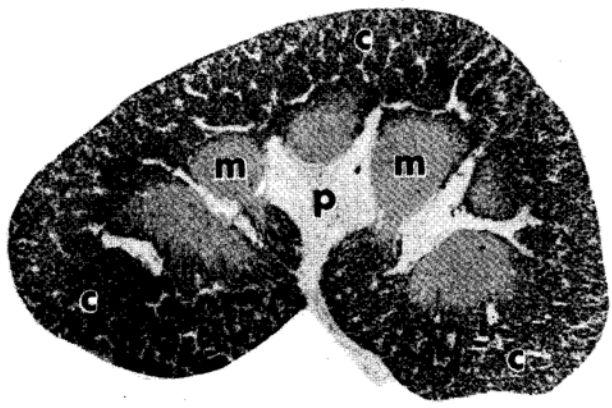

Fig. 4. Autoradiographic image of [³H]tryptamine binding to a 10-μ section of a rat kidney. Abbreviations: c, cortex; m, medulla; p, pelvis.

Whereas the affinity constants of the five nervous tissue regions appear to be similar, the affinity for choroid plexus was statistically significantly different from that in cortex. The nonchoroid affinities are very similar to values reported previously for homogenate binding studies (2.5–2.8 nM; Kellar and Cascio, 1982; Cascio and Kellar, 1983; Bruning and Rommelspacher, 1984; Wood *et al.*, 1984; Charlton *et al.*, 1985).

Binding to homogenates of bovine choroid plexus. In order to study the tryptamine choroid plexus binding site further, tissue from bovine brain was used for homogenate binding experiments. Saturation studies with [³H]tryptamine yielded a K_D of 13.5 nM ± 2.8 ($n = 4$), with a Hill slope of 1.04 and a capacity of 4.31 ± 0.08 pmol/mg of protein. Binding was inhibited by NaCl, with an IC_{50} of 43 mM; the guanine nucleotide guanylylimidodiphosphate had no effect on binding at concentrations of 300 μM. Pazos *et al.* (1985) recently reported the presence of a new type of serotonergic binding site in porcine choroid plexus. This site was labeled with several ligands, including [³H]serotonin (K_D = 6.5 mM) and [³H] mesulergine (K_D = 1.1 nM). In order to determine if this serotonergic site was equivalent to the [³H]tryptamine site labeled in bovine choroid plexus, competition studies were performed (table 3). These data indicate that the sites are not equivalent; serotonin has only a modest affinity (410 nM) to the bovine choroid plexus tryptamine site and drugs such as

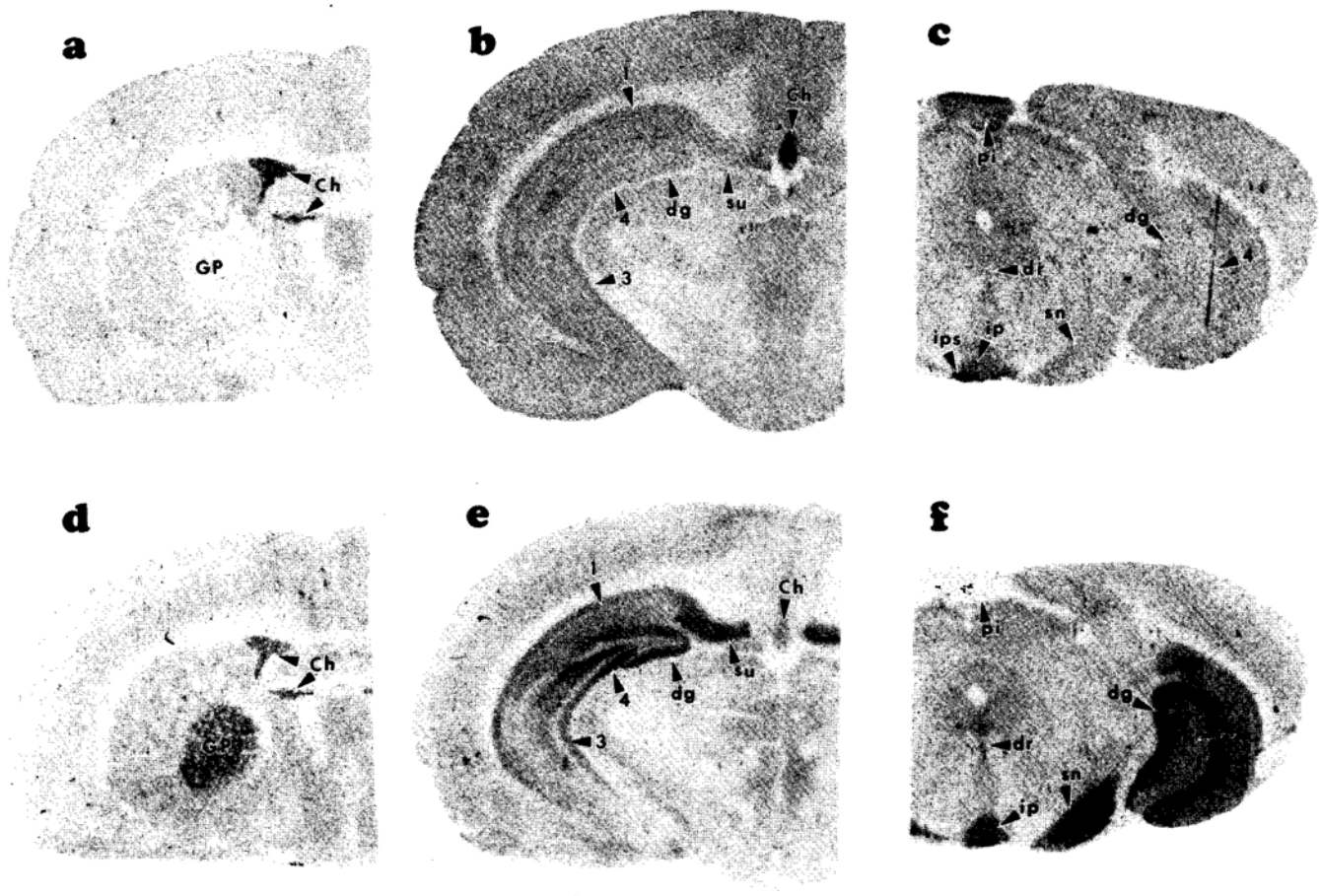

Fig. 5. Autoradiographic images of [³H]tryptamine (a–c) and [³H]serotonin (d–f) binding to adjacent coronal sections from three brain regions. Images represent total binding using 7.6 and 7.9 nM ligand concentrations, respectively; film was exposed for 47 days. Abbreviations are as in figure 3 legend, except: 1,3,4, CA1, CA3, CA4 regions of hippocampus; dg, dentate gyrus; ips, inner post subnucleus of the interpeduncular nucleus; pi, pineal body; su, subiculum.

TABLE 2

[^{3}H]Tryptamine autoradiography: saturation analysis in rat brain regions

Binding constants were calculated by computerized densitometry using ^{3}H standards. Values are mean (±S.E.) of determinations in four animals (cortex and choroid plexus) or two animals (others). Each determination used from six to nine concentrations of tryptamine; each data point was replicated at least three times. Cortex was from the frontoparietal region (laminae II–IV); corpus callosum was sampled in forebrain; others were averages taken over entire coronal sections of each region.

Region	K_D	B_{max}
	nM	*fmol/mg protein*
Choroid plexus	9.84* (±1.48)	1390 (±513)
Cortex	2.16 (±0.26)	154 (±38)
Hippocampus	1.51	168
Interpeduncular nucleus	2.48	478
Corpus callosum	2.51	24
Periaqueductal gray	2.86	88

* Different from cortex value; $P < .05$.

TABLE 3

[^{3}H]Tryptamine binding, bovine choroid plexus homogenate

Values are from single determinations except for tryptamine and serotonin in which $n = 4$ (±S.E.). Compounds with little or no activity inhibited binding by less than 10% at 1000 nM.

Compound[a]	K_i
	nM
Harmaline	1.0
Tryptamine	13.5 (±2.8)
5-Methyltryptamine	17.3
THBC	205
Serotonin	410 (±86)
Phenylethylamine	500
Dopamine	1400
d-Amphetamine	2130
l-Amphetamine	3560

[a] Little or no activity: melatonin; methysergide; ketanserin; mianserin; cinanserin; metergoline; mesulergine; spiperone; cyproheptadine; 5,6-dihydroxytryptamine; 5-hydroxytryptophan; fluoxetine; probenecid; mazindol; propranolol; phentolamine; phenoxybenzamine; and atropine.

methysergide, mianserin and mesulergine which displayed high affinity for the porcine choroid plexus serotonin site (Pazos *et al.*, 1985) showed no measurable affinity for the bovine choroid plexus tryptamine site (table 3).

In order to determine whether there is also a serotonergic site in bovine choroid plexus similar to that described in porcine choroid plexus, a saturation experiment with [^{3}H]serotonin was performed. [^{3}H]Serotonin labeled a site with a K_D of 13.4 nM and a B_{max} of 1.93 pmol/mg of protein. In competition experiments, tryptamine inhibited this binding with a K_i of 22 nM, and mesulergine with a K_i of 13 nM. These data are consistent with the presence in bovine choroid plexus of the new serotonergic site described for porcine choroid plexus (Pazos *et al.*, 1985) and in addition a separate site labeled by [^{3}H]tryptamine. These data do not rule out the possibility that [^{3}H]tryptamine may be labeling the serotonin site as well as a tryptamine site (a possibility which is supported by the higher capacity obtained for [^{3}H]tryptamine saturation experiments).

Densitometric analysis of rat choroid plexus binding sites. Saturation analysis of autoradiograms revealed that the tryptamine site in the rat choroid plexus appeared to be different from that in the rest of brain (table 2); homogenate binding studies with bovine choroid plexus (table 3) suggested that this choroid plexus site was also separate from the serotonergic site described by Pazos *et al.* (1985). Competition experiments were done to compare [^{3}H]tryptamine sites in rat cortex and choroid plexus and [^{3}H]tryptamine sites and [^{3}H]serotonin sites in

TABLE 4

Rat brain autoradiography: drug competition for [^{3}H]tryptamine and [^{3}H]serotonin binding in choroid plexus and cortex

Values represent K_i values in nanomolars; ni, no inhibition of binding observed at 1000 nM; >1000, some inhibition seen at 1000 nM but less than 20%; —, not tested. Each is the mean of determinations in two animals (except tryptamine; $n = 4$).

Drug	*vs.* [^{3}H]Tryptamine		*vs.* [^{3}H] Serotonin
	Cortex	Choroid	Choroid
Tryptamine	2.2	9.8	40
Serotonin	310	130	4.1
Kynuramine	11.4	ni	>1000
Phenylethylamine	260	1280	>1000
THBC	26	140	—
BOL	>1000	780	—
Ketanserin	ni	ni	200
Mianserin	ni	ni	<100
Methysergide	ni	ni	22
Mesulergine	ni	ni	9.4

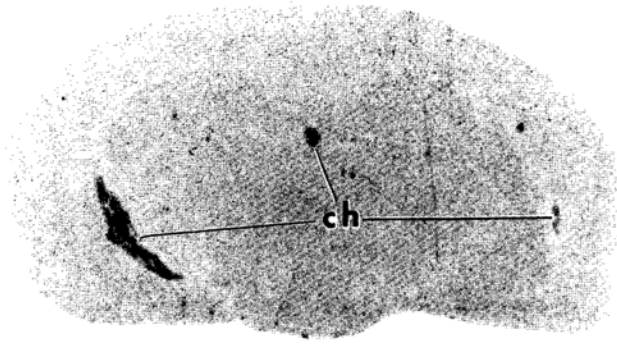

Fig. 6. Coronal rat brain section corresponding to figure 22 from the atlas of Paxinos and Watson (1982). Section was labeled with [^{3}H] tryptamine (3.1 nM) in the presence of 600 nM kynuramine. This is an adjacent section to figure 3E. ch, choroid plexus.

choroid plexus; results are presented in table 4. The tryptamine sites in cortex and choroid plexus show some similarities, but they can be distinguished by their different affinities for several compounds, including phenylethylamine, THBC, BOL and, most dramatically, the endogenous tryptamine-like compound kynuramine. Previous homogenate studies demonstrated that this compound and congeners have high affinity for [^{3}H]tryptamine sites in rat cortex (Charlton *et al.*, 1985); similar high affinity is seen here for rat cortex. However, essentially no inhibition of [^{3}H]tryptamine binding to choroid plexus was seen. Figure 6 presents these results graphically, showing [^{3}H] tryptamine binding in the presence of 600 mM kynuramine; only choroid plexus labeling can be detected. This section can be compared to figure 3E, which shows total [^{3}H]tryptamine binding to an adjacent section.

The pharmacological profiles of the tryptamine and serotonin sites in the rat choroid plexus shown in table 4 show these to be distinct sites. The affinity of serotonin for its own site is some 30 times that for the tryptamine site, and the serotonergic drugs ketanserin, mianserin, methysergide and mesulergine, which all have appreciable affinity for the serotonin site, have none for the tryptamine site.

Examined under a high-power light microscope, the grain patterns produced by these two ligands in the choroid plexus

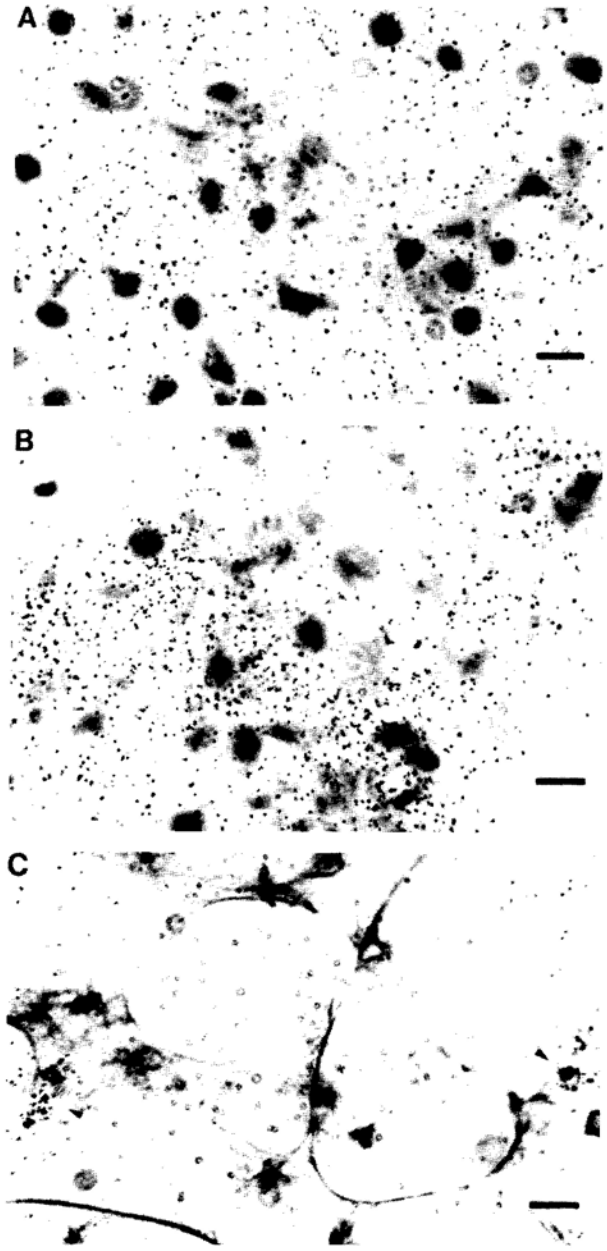

Fig. 7. Photomicrographs of coronal 10-μ section labeled with 3.1 nM [³H]tryptamine (A and C) or 3.9 nM [³H]serotonin (B). Emulsion-coated coverslips were glued to the slides and exposed for 45 days. Panels A and B are from the choroid plexus (3rd ventricle); panel C is from frontal cortex. Arrows point to clusters of grains. Bars = 10 m.

also appeared distinct. Figure 7A shows a typical grain distribution produced by [³H]tryptamine: relatively dense, but evenly distributed throughout the tissue. In figure 7B, the grain pattern produced by [³H]serotonin is seen in an adjacent section; the dense but uneven labeling was typical for this ligand, and contrasts with the relatively homogeneous pattern in figure 7A. Figure 7C shows [³H]tryptamine binding in the cortex of the same section as figure 7A; the clumps of grains seen here (arrows) may represent a synapse or cell body with a concentration of tryptamine binding sites.

Discussion

The recent reports of high-affinity brain binding sites for tryptamine by Kellar and others (Kellar and Cascio, 1982, 1986; Cascio and Kellar, 1982, 1983; Bruning and Rommelspacher, 1984; Wood *et al.*, 1984; Charlton *et al.*, 1985) have supported the role of this endogenous amine as a potential neurotransmitter or neuromodulator. In order to clarify tryptamine's potential role further, *in vitro* autoradiographic studies were undertaken. The findings reported here extend the previously reported autoradiographical studies (Perry *et al.*, 1982) and, in addition, demonstrate a unique tryptamine binding site in the choroid plexus. Another autoradiographic study has been reported in abstract form (McCormack *et al.*, 1983). These authors used different incubation conditions, and their reported affinity constants (tryptamine K_D = 13.00 nM; serotonin K_i = 1370 nM) were higher than presented here. Except for the lack of mention of choroid plexus binding, the general distribution reported by these authors is in substantial agreement with the current findings.

The initial characterization of [³H]tryptamine binding to rat brain sections involved kinetic, saturation and binding competition experiments, and demonstrated that [³H]tryptamine binding under *in vitro* autoradiographic conditions was similar to previously reported homogenate binding studies (Cascio and Kellar, 1982, 1983; Wood *et al.*, 1984; Bruning and Rommelspacher, 1984). The affinity and pharmacology of the sites appeared to be essentially the same in sections and homogenates (fig. 2; table 1); tryptamine was the most potent compound at these sites (K_D = 3.51 nM) and serotonergic compounds had only modest or no affinity.

Computerized densitometry allowed detailed analysis of binding characteristics to individual regions. The interpeduncular nucleus had by far the highest density of tryptamine sites, several-fold higher than the next highest regions, the cortex and hippocampus. This result suggests that this nucleus may be an important region for further pharmacological and electrophysiological experiments designed to clarify the role of tryptamine in brain function. The apparently similar affinities in the five nervous tissue regions examined indicates homogeneity of the tryptamine sites. The most striking result from the quantitative regional analysis was the unexpected finding that the choroid plexus affinity for tryptamine was significantly lower than the rest of the brain; this led to experiments to further clarify the characteristics of this choroid site.

The overall distribution of binding sites paralleled that reported in homogenate studies (Cascio and Kellar, 1983). Rostral structures, such as the caudate putamen, cortex and hippocampus, had high levels of binding. Binding was widely distributed throughout the entire brain, and most gray matter showed at least modest levels of specific binding. Only very low levels were detected in white matter, although specific binding was present; see, for instance, table 2, showing detectable levels of specific binding to the corpus callosum. [³H]Tryptamine binding, although ubiquitous, demonstrated considerable heterogeneity. A number of regions which demonstrated high levels of [³H]tryptamine binding could be described as receptor "hot spots"; that is, they have been reported as regions of dense labeling for a number of different neurotransmitter receptor ligands. In regions with well-defined cytoarchitecture, binding was often noticeably higher in those parts which are enriched in nerve terminals, *e.g.* lamina I of the cortex and the strata oriens and moleculare-laconosum of the hippocampus. This distribution is consistent with the role of a neuromodulator or neurotransmitter. However, the ubiquitous nature of the tryptamine sites precludes any simple conclusions from being drawn

about the physiological role of tryptamine solely on brain localization.

The highest levels of [^{3}H]tryptamine binding outside of the choroid plexus were seen in the interpeduncular nucleus, especially the inner post subnucleus (fig. 3F; table 2). This nucleus is in the limbic midbrain, and is considered a part of the ventral tegmental area, along with the raphe nuclei; it receives serotonergic input from the dorsal raphe nucleus (Angevine and Cotman, 1981). Along with the habenula, it contains the highest levels of choline acetyltransferase in the brain, and there is dense cholinergic and substance P innervation from the habenula to the interpeduncular nucleus by way of the fasiculus retroflexus (or habenulo-peduncular tract) (Angevine and Cotman, 1981). Considerable serotonin binding was also detected in this nucleus. The presence of a large number of tryptamine sites in a region with dense cholinergic innervation suggests a potential interaction of tryptamine with acetylcholine. Jones (1982a) has described the attenuation of certain cholinergic responses with subthreshold applications of tryptamine.

Because of the structural similarity to serotonin, the possibility that tryptamine sites play some role in serotonergic transmission must be considered. For instance, [^{3}H]tryptamine could be labeling some kind of serotonin uptake site, or autoreceptor, or perhaps a subclass of postsynaptic serotonin receptors. These possibilities were addressed by comparing the localizations and the pharmacological profiles of tryptamine and serotonin sites. Several publications describe the autoradiographic localization of various classes of serotonergic sites using a variety of ligands, including [^{3}H]LSD, [^{125}I]LSD, [^{3}H] serotonin, [^{3}H]-8-hydroxy-N,N-dipropyl-2-aminotetralin and [^{3}H]spiperone (Young and Kuhar, 1980; Meibach *et al.*, 1980; Palacios *et al.*, 1981, 1983; Biegon *et al.*, 1982; Deshmukh *et al.*, 1983; Marcinkiewicz *et al.*., 1984; Nakada *et al.*, 1984). The current findings using [^{3}H]serotonin (see fig. 5, d–f) substantially support these previous studies and demonstrate that the general serotonin site distribution shows many similarities to that for [^{3}H]tryptamine. For instance, relatively high levels of binding for both ligands was detected in the choroid plexus, cortex, caudate putamen, nucleus accumbens, amygdala, olfactory tubercle, hippocampus, interpeduncular nucleus, superior colliculus and central gray region of the pons.

Other brain regions demonstrated quite different patterns for serotonin and tryptamine sites. For instance, serotonin binding was much higher than tryptamine binding in the globus pallidus, the substantia nigra, the preoptic area and several raphe nuclei, especially the dorsal raphe, (figs. 5, c and f; Biegon *et al.*, 1982). The raphe nuclei are of particular interest because these nuclei contain cell bodies of serotonergic neurons. The median raphe nucleus has also been suggested as a possible tryptamine containing nucleus (Jones, 1982a). If [^{3}H]tryptamine was labeling some form of uptake or autoreceptor site, this would be a location in which dense labeling would be predicted. However, only marginal levels of [^{3}H]tryptamine binding were seen in the raphe nuclei (moderate binding in the dorsal raphe). In contrast, several regions showed relatively high levels of tryptamine binding, but very little serotonin binding. Most notable is the cerebellum; gray matter showed moderate tryptamine binding throughout (figs. 3, H–K) but virtually no serotonin binding (Biegon *et al.*, 1982; Meibach *et al.*, 1980). Other such regions include the medial geniculate nucleus, the habenula, the CA3 field of the hippocampus and the pineal body.

Furthermore, a closer examination of the labeling within some of the apparently similar regions often shows significant differences between tryptamine and serotonin. For example, serotonin sites in the cortex are least dense in lamina I (Meibach *et al.*, 1980; Biegon *et al.*, 1982), whereas this is the highest layer for tryptamine binding (figs. 3 and 5). The serotonin labeling of the interpeduncular nucleus is described as being "essentially uniform" (Biegon *et al.*, 1982) (fig. 5f), whereas clear heterogeneity is apparent in the tryptamine labeling of this nucleus (figs. 3F and 5c). Serotonin binding in the hippocampus shows distinct regional variation, (figs. 5, e and f; Biegon *et al.*, 1982; Meibach *et al.*, 1980; Marcinkiewicz *et al.*, 1984). In contrast, tryptamine labeling showed only minor differences among the fields (figs. 3, E and F and 5, b and c). Thus, with higher anatomical resolution possible with *in vitro* autoradiography one can see that some of the apparent similarities between the two receptor distributions are not as striking.

The pharmacological profiles of [^{3}H]serotonin and [^{3}H]tryptamine binding to rat brain sections also serve to distinguish the tryptamine site from any known serotonergic site. Table 1 shows that drugs such as LSD, which has high affinity for 5-HT_{1a} and 5-HT_2 sites, and spiperone, which has high affinity for 5-HT_2 sites, have no affinity for tryptamine sites. Serotonin itself has only modest affinity for tryptamine sites. Other serotonergic drugs, such as mianserin, ketanserin, methysergide and mesulergine (which has high affinity for the putative 5-HT_{1c} site; Pazos *et al.*, 1985) do not bind to the tryptamine site. Furthermore, several compounds which do have high affinity for tryptamine sites (kynuramine, phenylethylamine, THBC, tryptamine and 5-methyltryptamine; tables 1 and 4) have little or no affinity at serotonin sites. Fluoxetine, which has been shown to inhibit both serotonin and tryptamine uptake in brain tissue (Jones, 1982a), had no affinity for the tryptamine binding site in brain (not shown) or choroid plexus (table 3).

In his review of the potential roles of brain tryptamine, Jones (1982a) stresses that many of the effects of tryptamine are opposite to those of serotonin, and suggests that these two amines may mediate functionally opposite neuronal pathways. The existence of a separate class of "tryptaminergic" neurons, projecting rostrally from the median raphe nucleus, has been suggested but not yet proved (Jones, 1982a). It is also possible that tryptamine is produced in serotonergic neurons, or perhaps in a subset of these neurons, and is released from these neurons to act on tryptamine sites. The considerable overlap of tryptamine sites with serotonin sites, and with regions high in serotonin terminals (even when lacking serotonin receptors), would seem to support this possibility. Tryptamine may represent a product of an alternate synthetic pathway in "serotonergic" neurons, produced and/or released instead of serotonin in response to certain specific stimuli, whereas serotonin is the dominant form under normal circumstances. Tryptamine may also act as a "modulator" of serotonin function, either released from its own or from serotonergic nerves (Jones, 1982a). Evidence against the location of functional tryptamine in serotonergic neurons is the finding that [^{3}H]tryptamine binding is unchanged after lesioning with 5,7-dihydroxytryptamine or 6-hydroxydopamine (Cascio and Kellar, 1986). This finding also argues against the localization of tryptamine sites on serotonergic or catecholaminergic neurons. Whereas tryptamine

clearly labels a distinct population of sites, the physiological evidence of interactions between serotonin and tryptamine (Jones, 1982a,b,c) and the overlap in distribution of receptor fields presented here strongly suggest that these two amines are somehow functionally related, at least in some areas in the brain.

The choroid plexus consistently showed the highest levels of [³H]tryptamine binding of all regions. Previous studies demonstrated intense labeling of this structure with serotonergic ligands, including [³H]LSD (Meibach *et al.*, 1980) and [³H] serotonin (Young and Kuhar, 1980; Biegon *et al.*, 1982). Recently, Pazos *et al.* (1985) reported that the serotonergic site in porcine and rat choroid plexus displayed a distinct pharmacological profile, and proposed that these be called 5-HT_{1c} sites. This site is labeled by [³H]serotonin, [³H]mesulergine and [³H] LSD; the latter ligand labels almost twice as many total sites in procine choroid plexus as the other two, a fact which the authors cannot explain (Pazos *et al.*, 1985).

In order to determine whether the choroid tryptamine site was identical to the choroid serotonin site (5-HT_{1c}), pharmacological profiles were determined in rat and bovine choroid. Competitive binding studies with [³H]tryptamine using bovine choroid homogenates (table 3) revealed a profile similar to the tryptamine site in brain but quite unlike any serotonin site, including the 5-HT_{1c} site. For instance, mesulergine had no affinity *vs.* [³H]tryptamine, whereas [³H]mesulergine labels porcine choroid with a $K_D = 1.1$ nM (Pazos *et al.*, 1985). A wide variety of other compounds, including serotonergic, adrenergic and cholinergic drugs, also did not bind to the choroid tryptamine site (table 3). Unfortunately, LSD was not tested against this site; it is possible that [³H]LSD labels both the 5-HT_{1c} site and the tryptamine site in choroid, which would explain why this ligand labels twice the number of sites as [³H]serotonin. Evidence to support this possibility comes from autoradiographic experiments (not shown) which found that 10^{-5} M LSD inhibited [³H]tryptamine binding to rat choroid completely, whereas the same concentration of LSD showed essentially no inhibition of [³H]tryptamine binding to sagittal brain slices (table 1). Densitometric analysis revealed that the tryptamine site in the rat choroid not only differed from the serotonin 5-HT_{1c} site, but from the tryptamine site in the rest of the brain. The endogenous compound kynuramine gave the clearest distinction between the two tryptamine sites; it potently inhibited [³H]tryptamine binding to brain, without affecting choroid plexus binding (fig. 6). In keeping with previous multiple receptor systems, I propose that the tryptamine site in brain be referred to as the T-1 site, and that the choroid plexus tryptamine site be referred to as the T-2 site.

The function of the T-2 site, as well as the 5-HT_{1c} site, is unknown. Because the choroid plexus acts to secrete and filter CSF (Netsky, 1975), it is reasonable to speculate that these sites may be involved somehow in regulating these processes. The choroid plexus is known to be innervated by several types of nerves, including serotonergic nerves (Mollgard and Wiklund, 1979; Chan-Palay, 1976; Aghajanian and Gallager, 1975; Moskowitz *et al.*, 1979). It has been suggested that the serotonergic sites in the choroid plexus represent postsynaptic sites, presumably located on either the secretory epithelial cells or possibly on the blood vessels; these sites may serve to mediate neural control of the CSF secretion by the choroid plexus (Aghajanian and Gallager, 1975; Chan-Palay, 1976; Moskowitz *et al.*, 1979; Meibach *et al.*, 1980). It is possible that at least some of the amine measured in these serotonergic nerves was not serotonin, but a "closely related indoleamine" (Aghajanian and Gallager, 1975; Richards *et al.*, 1973). If there are tryptamine neurons innervating the choroid plexus, the T-2 tryptamine sites may also be serving as post-synaptic sites involved in regulation of CSF secretion. The T-2 sites probably do not represent reuptake sites because fluoxetine, a drug which blocks reuptake of both serotonin and tryptamine (Jones, 1982a), did not affect T-2 binding. Also, transmitter reuptake is generally accepted to be sodium-dependent and temperature-dependent. T-2 binding, in contrast, did not require sodium ions and in fact was inhibited rather potently by NaCl; and binding was superior at 4°C compared to 37°C.

Choroid plexus binding sites may also act as hormonal receptors, sensing the presence of compounds in the CSF. These receptors may be on nerve endings [*i.e.*, the "sensory nerves" described by Netsky (1975)] or directly on epithelial cells. Such a possibility has been considered for serotonin; many of the serotonergic nerves associated with the ventricular system have terminals in the ependymal cells instead of the choroid plexus itself, and these may serve as neurosecretory nerves (Aghajanian and Gallager, 1975). Jaeger *et al.* (1983) describe "CSF contacting neurons which may possess neurosecretory as well as neuroreceptor function," and note that these particular neurons would to be likely to secrete trace amines such as tryptamine, phenylethylamine and tyramine (Jaeger *et al.*, 1983). The great density of the T-2 sites in choroid plexus and their differences from the presumably neuronal T-1 sites in brain makes this possible function as receptors for CSF hormones an attractive hypothesis.

Photomicrographs of [³H]tryptamine binding to choroid plexus (fig. 7A) revealed a surprisingly homogeneous distribution of grains, with no apparent association of grains with any apparent cell type (such as the secretory epithelial cells) or structural feature (such as blood vessels). In contrast, [³H] tryptamine binding in rat cortex (fig. 7C) demonstrated characteristic clusters of grains, possibly representing synapses. [³H]Serotonin binding to choroid plexus (fig. 7B) appeared to be more heterogeneous than [³H]tryptamine. In neither case, however, could binding be associated clearly with any structural feature of the organ.

The existence of a second tryptamine binding site suggests the possibility that this site might be found in other tissues, as Pazos *et al.* (1985) indicate that the 5-HT_{1c} site appears to be present in cerebral cortex. As yet there does not appear to be a drug specific for the T-2 site which could be used to readily distinguish their existence (although kynuramine could be used to selectively block T-1 labeling). The apparent high affinity ($K_i = 1.0$ nM) of harmaline at bovine choroid plexus tryptamine sites is interesting in this regard, however, because previous reports indicate that harmaline's affinity for the T-1 site is at least an order of magnitude lower (Bruning and Rommelspacher, 1984). No evidence for multiple tryptamine binding sites in brain tissue has been reported, and none was seen here. However, many other tissues demonstrate tryptamine binding sites (Bruning and Rommelspacher, 1984). The kidney would seem to be a likely source to look for T-2 sites, as the CSF filtration and secretion of the choroid plexus is similar in nature to the filtration and secretion functions of the kidney. Rat kidney slices showed dense labeling in cortical regions, with light labeling in the medulla and none in the pelvis and ureter (fig. 4). Bruning and Rommelspacher (1984) showed that the

affinity and pharmacological profile of the tryptamine site in rat kidney homogenates were very similar to rat cerebral cortex, suggesting that the kidney contains T-1 sites only. However, their results did not rule out T-2 sites (for instance, neither kynuramine, phenylethylamine or LSD was tested), and the possibility that cortical and medullary binding might differ was not tested.

The pineal body, which synthesizes the hormone melatonin (N-acetyl-5-methoxytryptamine) was also found to contain tryptamine (but not serotonin) binding sites (figs. 5, c and f). Analysis of a single autoradiography experiment (not shown) indicated that the apparent affinity of the pineal sites was noticeably lower than the rest of the brain, suggesting the possible presence of T-2 sites of this organ.

In summary, [^{3}H]tryptamine was demonstrated to label sites in rat brain sections with the same characteristics as described previously for brain homogenate binding. These sites were widely distributed throughout brain gray matter; rostral structures, especially in the limbic brain, showed particularly high levels. Labeling was also detected in specific structures in the thalamus, hypothalamus, midbrain, pons, medulla and cerebellum. Although several major similarities to the pattern of serotonin receptor distribution were apparent, the distribution and pharmacology of the tryptamine site was clearly distinct from known serotonin receptors. A second tryptamine site, called T-2, was detected in the choroid plexus; this site was distinct from both the serotonin site in the choroid plexus and the tryptamine site in the rest of the brain.

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