Chronic Effects of Imipramine and Lithium on 5-HT Receptor Subtypes in Rat Frontal Cortex, Hippocampus and Choroid Plexus: Quantitative Receptor Autoradiographic Analysis

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Accepted April 6, 1989

Abstract—The effects of chronic treatment with imipramine or lithium on serotonin (5-HT) receptor subtypes were analyzed in the frontal cortex, hippocampus and choroid plexus of rat brain by quantitative receptor autoradiographic procedures, using radioligands [\(^3\)H]-5-HT, [\(^3\)H]-8-hydroxy-2-(di-n-propylamino)tetratin ([\(^3\)H]-8-OH-DPAT), [\(^1\)25I]-iodocyanopindolol ([\(^1\)25I]-CYP), [\(^3\)H]-mesulergine and [\(^1\)25I]-7-amino-8-iodo-ketanserin ([\(^1\)25I]-ketanserin) or [\(^3\)H]-spiperone. Chronic i.p. administration of imipramine (20 mg/kg/day for 21 days) decreased the densities of 5-HT\(_{1A}\), 5-HT\(_{1B}\), 5-HT\(_{1C}\) and 5-HT\(_2\) sites in the frontal cortex, hippocampus and choroid plexus. Lithium (2 mEq/kg/day for 21 days) also decreased the densities of 5-HT\(_1\), 5-HT\(_{1A}\), 5-HT\(_{1C}\) and 5-HT\(_2\) sites in the frontal cortex, and the densities of those including 5-HT\(_{1A}\) sites in the hippocampus and choroid plexus. Imipramine and lithium very markedly decreased the density of 5-HT\(_{1C}\) sites in the choroid plexus. We propose that methods employing quantitative receptor autoradiographic analysis can be used to characterize and understand the local effects of these drugs on 5-HT receptor subtypes.

Since two main types of central serotonin (5-HT) receptors have been characterized: 5-HT\(_1\) receptors labeled by [\(^3\)H]-5-HT and 5-HT\(_2\) receptors labeled by [\(^3\)H]-spiperone (1). 5-HT\(_1\) receptors have been shown to be heterogenous (2), and three distinct subtypes of the 5-HT\(_1\) receptors have been identified (3). The 5-HT\(_{1A}\) sites are labeled with [\(^3\)H]-8-hydroxy-2-(di-n-propylamino)tetratin ([\(^3\)H]-8-OH-DPAT) and display nanomolar affinity for 8-OH-DPAT (4). The 5-HT\(_{1B}\) sites labeled with (−)[\(^1\)25I]-iodocyanopindolol ([\(^1\)25I]-CYP) are present in rat and mouse brains (5). The 5-HT\(_{1C}\) sites labeled with [\(^3\)H]-mesulergine are densely distributed in the choroid plexus and cortex and display nanomolar affinity for 5-HT, mesulergine, methysergide and mianserin (3, 6). Although all 5-HT\(_1\) receptors can be labeled by [\(^3\)H]-5-HT, each of the known subtypes displays a unique pharmacological profile. In addition, quantitative autoradiographic mapping of these 5-HT receptor subtypes has been performed in the mammalian CNS (7–10).

Previously, we have reported that chronic but not acute treatment with many tricyclic antidepressants reduces the density of 5-HT receptors in rat brain membranes (11). Most tricyclic antidepressants reduce the density of 5-HT\(_2\) receptors in the cerebral cortex, although with the exception of imipramine, they have no effect on 5-HT\(_1\) receptors (12, 13). Most recently, we have observed that imipramine reduces the maximum number of binding sites (B\(_{max}\)) for 5-HT\(_{1A}\) sites, but not for 5-HT\(_{1B}\) sites in either the frontal cortex or the hippocampus (14). On the other hand, long-term administration of lithium, an effective drug for manic-depressive illness, also reduces the density of not only 5-HT\(_1\) and 5-HT\(_2\) receptors in the hippocampus but also 5-HT\(_2\) receptors in the frontal cortex (15, 16). Most recently, our studies have indicated that lithium reduces the B\(_{max}\) for postsynaptic 5-HT\(_{1A}\) sites in the hippocampus, but not in the frontal cortex (14).
In the present study, we have utilized an autoradiographic technique on rat brain slices to further extend our previous biochemical findings with imipramine and lithium, and assessed the effects of chronic administration of both drugs on 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$ and 5-HT$_{2}$ sites in the frontal cortex, hippocampus and choroid plexus.

**Materials and Methods**

**Animals and treatments:** Male Wistar rats (230–270 g, Japan Lab. Animals) were used for this study. Animals were kept for at least 7 days in a controlled environment (22±2°C, 55±5% humidity, lights on 7 a.m.–7 p.m., food and water ad libitum) before being used. Imipramine (20 mg/kg, i.p.) or LiCl (2 mEq/kg, i.p.) were administered once daily at 10 a.m. for 21 consecutive days. Maximum serum lithium concentration after this treatment was 0.8±0.2 mEq/ml (n=5). After pentobarbital anesthesia (35 mg/kg) at 24 hr after the last injection of both drugs, the animals were sacrificed by intracardial perfusion with 0.1% formalin in phosphate-buffered saline. The brains were removed and frozen in Tissue-Tek OCT compound by dry-ice cooled acetone, and 10 μm coronal or sagittal sections were cut using a Reichert Histostat microtome 855. The sections were mounted on albumin-coated microscope slides and stored at -20°C until use within a few days.

**Autoradiographic procedures:** The autoradiographic procedures were carried out by slightly modifying the methods described by Pazos and Palacios (7, 8). Incubation conditions for each radiolabeled ligand, displacer, concentration of ligand, including buffer solution, incubation temperature and time, washing buffer and washing water are summarized in Table 1. The brain sections were incubated with each radiolabeled ligand in the presence (the nonspecific binding) or absence (the total binding) of excess displacers. After the washing period, tissues were dried under a cold air stream and the dry slides were exposed to Hyperfilm-[³H] (Amersham) for 3 months at 4°C for [³H]-labeled ligands and 4 days at -80°C for [¹²⁵I]-labeled ligands. The film was developed and then subjected to color-coded reconstruction and quantification with a computerized receptor autoradiography image analysis system (RAS-R1000, Amersham). Specific images were obtained by subtracting the nonspecific image from the total binding image.

**Calibration:** The tissue sections were coexposed with calibrated [³H] micro-scales or [¹²⁵I] micro-scales standards (Amersham). Under these conditions, the linear range of both micro-scales for the Hyperfilm-[³H] was confirmed to be about 0.07–1.10 optical density (OD) units. Calibration of both micro-scales for tissue equivalent radioactivity concentrations were performed according to Geary et al. (17). Increasing doses (10–500 μCi, i.v.) of each radiolabeled ligand were administered to 5 rats each; the animals were sacrificed at 30–50 min post-injection; and then their brains were rapidly removed. Brains were hemisected in the mid-sagittal plane. One half-brain was frozen in Tissue-Tek OCT compound and cut at serial 10 μm sagittal sections; afterwards, autoradiography was performed as described above, and regional ODs were determined by comparison with the ODs of micro-scales standards. The other half-brain was regionally dissected into the frontal cortex, hippocampus and choroid plexus. These dissected regions were homogenized in deionized H$_2$O; [³H] radioactivity in the aliquots was counted in 10 ml Aquasol scintillation cocktail (Du Pont) by a liquid scintillation spectrometer; and [¹²⁵I] radioactivity in the aliquots was counted by a gamma-meter. Protein concentrations in the same aliquots were assayed by the Lowry method (18). Radioactivity concentrations in these dissected tissues were converted to nCi/mg protein. Calibration curves for tissue equivalent radioactivity concentrations (nCi/mg protein) were prepared by plotting regional tissue radioactivity concentrations against regional ODs of the other hemisphere. An OD measured in a region was interpolated to a value expressed as nCi/mg protein by each calibration curve fitting. Specific bindings were determined by the following relationship: fmol/mg protein= (XnCi/mg protein) x (fmol/VnCi), where X= tissue equivalent value over the region being studied and V= specific activity of the radiolabeled ligand.

**Autoabsorption:** To estimate the differences...
Table 1. Incubation conditions used for the labeling of 5-HT receptor subtypes

| Subtype | Ligand     | Displacer | Preincubation protocol | Buffer                              | Incubation time | Washing Buffer | Washing Water |
|---------|------------|-----------|------------------------|-------------------------------------|-----------------|----------------|---------------|
| 5-HT₁  | [³H]-5-HT  | 5-HT (1)  | 10 min (25°C)          | 0.17 M Tris-HCl-4 mM CaCl₂-0.01% ascorbic acid-10 μM niatamide (pH 7.8) | 60 min (25°C)   | 2 × 10 min     | 2 × 10 min     |
|         | (20)       |           |                        |                                     |                 | (4°C)          | (4°C)          |
| 5-HT₃A | [³H]-8-OH-DPAT | 5-HT (1) | 10 min (25°C)          | same as above                       | 60 min (25°C)   | 2 × 10 min     | 2 × 10 min     |
|         | (2)        |           |                        |                                     |                 | (4°C)          | (4°C)          |
| 5-HT₃B | [¹²⁵I]-CYP  | 5-HT (1)  | none                   | same as above                       | 30 min (25°C)   | 2 × 10 min     | 2 × 10 min     |
|         | (0.17)     |           |                        |                                     |                 | (4°C)          | (4°C)          |
| 5-HT₃C | [³H]-mesulergine | 5-HT (1) | none                   | 0.17 M Tris-HCl (pH 7.7)             | 120 min (25°C)  | 2 × 10 min     | 2 × 10 min     |
|         | (10)       |           |                        |                                     |                 | (4°C)          | (4°C)          |
| 5-HT₃E | [¹²⁵I]-ketanserin | 5-HT (100) | 15 min (25°C)          | same as above                       | 60 min (25°C)   | 2 × 10 min     | 2 × 10 min     |
|         | (0.17)     |           |                        |                                     |                 | (4°C)          | (4°C)          |
| 5-HT₃E | [³H]-spiperone | mianserin | none                   | 0.17 M Tris-HCl-1 mM MgCl₂-2 mM CaCl₂-5 mM KCl-120 mM NaCl (pH 7.7) | 30 min (25°C)   | 2 × 10 min     | 2 × 10 min     |
|         | (5)        | (1)       |                        |                                     |                 | (4°C)          | (4°C)          |
in the autoabsorption (quenching) of the low energy of $[^3H]$ emissions between gray and white matter, we employed the procedures of chloroform extraction as described by Geary et al. (17). Tissues used were from the calibration experiments described above. Adjacent sections were either untreated or extracted in chloroform (500 ml) for 0.5–2 min, since all $[^3H]$ ligands were apparently insoluble in chloroform within this time. Sections were exposed for 3 months to Hyperfilm-$[^3H]$. Then the sections were scraped off the coverslips, weighed and counted in 2 ml Aquasol scintillation cocktail by a liquid scintillation spectrometer. Chloroform extraction was confirmed by monitoring changes in section weight and $[^3H]$ content. All chloroform extracted OD values for frontal cortex, hippocampus and choroid plexus were higher than OD values from their corresponding unextracted sections. The percent increases in OD values (mean±S.D., n=3) due to chloroform extraction over the unextracted value for each $[^3H]$ ligand image were as follows: (frontal cortex, hippocampus, choroid plexus=) 22.3±5.6, 31.3±6.2, 66.9±21.3 for $[^3H]$-5-HT; 20.1±6.3, 23.5±8.6, 36.6±16.6 for $[^3H]$-8-OH-DPAT; 35.3±5.1, 26.1±6.6, 72.1±18.1 for $[^3H]$-mesulergine; 37.3±8.5, 18.3±2.2, 13.5±7.3 for $[^3H]$-spiperone, respectively. These values were used in order to calculate correction factors for various brain regions.

**Competition and saturation analyses:** In competition studies, consecutive sections were incubated with different nanomolar radiolabeled ligands in the presence of increasing concentrations (1 nM–10 μM) of each displacer. Saturation experiments were carried out with concentrations of 0.1–50 nM of the different radiolabeled ligands in the presence of corresponding micromolar concentrations of displacer. Competition and saturation experiments were performed in triplicate and analyzed by non-linear computer-assisted curve fitting. $K_i$ values of displacer for radiolabeled ligand binding sites were estimated from the relationship: $K_i = IC50/(1+S/K_d)$, where $K_i$=inhibition constant of displacer, $IC50$=concentration of displacer that causes 50% inhibition, $S$=concentration of radiolabeled ligand and $K_d$=equilibrium dissociation constant.

Brain areas were identified and named using the rat brain atlas of Palkovits and Brownstein (19). The frontal cortical and hippocampal areas were identified following the rat brain dissection of Glowinski and Iversen (20). The choroid plexus totaled the areas of the lateral, third and fourth ventricles.

**Statistics:** Each structure was measured from at least three sections per animal (15–20 total determinations in 5 animals). Control and treated rats were compared using a two-tailed Student’s t-test.

**Drugs:** $[^3H]$-5-HT (14.5 Ci/mmol), $[^3H]$-8-OH-DPAT (206 Ci/mmol), $[^125I]$-CYP ($\sim$2000 Ci/mmol), $[^3H]$-mesulergine (83.6 Ci/mmol), $[^125I]$-ketanserin ($\sim$2000 Ci/mmol) and $[^3H]$-spiperone (17 Ci/mmol) were obtained from Amersham. 5-HT, imipramine hydrochloride and LiCl were purchased from Sigma. 8-OH-DPAT and mianserin were purchased from Research Biochemicals. Other compounds were of the highest analytical grade available.

**Results**

Pharmacological characterization of 5-HT receptor subtypes in three brain areas: The competition profile of displacers for radiolabeled ligands selectively labeling each 5-HT receptor subtype was analyzed by quantitative receptor autoradiography. The competition curves were sigmoidal in a monophasic manner (a one-binding site model) with Hill slopes of $\sim$1, with the exception of $[^125I]$-ketanserin that showed a clearly biphasic manner (a two-binding site model) (data not shown). The $K_i$ values obtained from the analysis of both the competition curves and the saturation experiments are shown in Table 2. $[^3H]$-5-HT binding to the hippocampus and choroid plexus was inhibited in the nanomolar range by 5-HT, while in the frontal cortex, 5-HT showed slightly lower affinity for these binding sites. 5-HT$_{1A}$ and 5-HT$_{1B}$ sites, which were labeled selectively by $[^3H]$-8-OH-DPAT and $[^125I]$-CYP, respectively, had the highest affinity for each displacer in the hippocampus and choroid plexus was inhibited in the nanomolar range by 5-HT, while in the frontal cortex, 5-HT showed slightly lower affinity for these binding sites. 5-HT$_{1A}$ and 5-HT$_{1B}$ sites, which were labeled selectively by $[^3H]$-8-OH-DPAT and $[^125I]$-CYP, respectively, had the highest affinity for each displacer in the hippocampus, followed by the frontal cortex, while the affinities in the choroid plexus were very low. In contrast, the affinity of 5-HT$_{1C}$ sites labeled selectively by $[^3H]$-mesulergine in the choroid plexus was...
Table 2. Affinities (K_i) of displacers for radiolabeled ligands to rat brain areas measured by quantitative autoradiography

| Subtype   | Ligand          | Displacer | Brain area                  |
|-----------|-----------------|-----------|------------------------------|
| 5-HT_1    | [³H]-5-HT       | 5-HT      | Frontal cortex | Hippocampus | Choroid plexus |
| 5-HT_1A   | [³H]-8-OH-DPAT  | 5-HT      | 21.3±8.6               | 5.8±1.3     | 5.3±2.2       |
|           | (8-OH-DPAT)     |           |                             |             |               |
| 5-HT_1B   | [¹²⁵I]-CYP      | 5-HT      | 18.8±6.3                | 4.1±1.6     | 1060±150      |
| 5-HT_2C   | [³H]-mesulergine| 5-HT      | 1820±210               | 3300±310    | 13.3±6.1      |
| 5-HT_3    | [¹²⁵I]-ketanserin| 5-HT      | 15.9±4.2               | 1520±160    | 1710±150      |
|           |                 | mianserin | 1220±130               | >5000       | >5000         |
| 5-HT_4    | [³H]-spioperone |           | 3.6±0.6                | 660±50      | 810±60        |

Values are expressed as K_i (nM), the mean±S.E.M. from triplicate determinations of 3 experiments. Where two values are given, they correspond to the high and low affinity sites.

Table 3. Effects of long-term administration of imipramine and lithium on the maximal densities of 5-HT receptor subtypes in the frontal cortex, hippocampus and choroid plexus from rat brains

| Drug      | Brain region      | [³H]-5-HT (20 nM) | [³H]-8-OH-DPAT (2 nM) | [¹²⁵I]-CYP (0.17 nM) | [³H]-mesulergine (10 nM) | [¹²⁵I]-ketanserin (0.17 nM) | [³H]-spioperone (5 nM) |
|-----------|-------------------|-------------------|-----------------------|---------------------|--------------------------|-----------------------------|------------------------|
| Control   | F. cortex         | 1170±89           | 365±52                | 403±64              | 635±63                   | 621±32                     | 1300±202               |
|           | Hippocam.         | 2996±350          | 783±23                | 1185±192            | 105±5                    | 72±15                      | 110±36                 |
|           | C. plexus         | 3328±502          | 36±3                  | ND                  | 182±42                   | 37±7                       | 7±1                    |
| Imipramine| F. cortex         | 482±64**          | 181±23*               | 357±33              | 282±57*                  | 219±11**                   | 487±101**              |
|           | Hippocam.         | 1221±108**        | 296±48**              | 1137±140            | 65±9*                    | 21±6*                      | 33±13*                 |
|           | C. plexus         | 1346±125**        | 11±1*                 | ND                  | 138±46***                | 14±3*                      | 2±1*                   |
| Lithium   | F. cortex         | 604±72*           | 338±35                | 311±48              | 317±66*                  | 281±27*                    | 621±111*               |
|           | Hippocam.         | 1112±234**        | 271±41***             | 954±101             | 73±6*                    | 35±6*                      | 46±17*                 |
|           | C. plexus         | 1165±216**        | 15±2**                | ND                  | 108±13***                | 17±5*                      | 2±1*                   |

Incubation conditions of slide-mounted tissue sections are shown in Table 1. Values are the mean±S.E.M. of 5 animals. *P<0.05, **P<0.01, ***P<0.001 vs. Control. ND: Not determined.
higher than in either the frontal cortex or the hippocampus. 5-HT receptors labeled selectively by \([^{125}\text{I}]\text{-ketanserin}\) or \([^{3}\text{H}]\text{-spiperone}\) had the highest affinity for each displacer in the frontal cortex, followed by the choroid plexus, while those in the hippocampus had very low affinity.

**Effect of drugs on 5-HT receptor subtypes:** Imipramine (1 \(\mu\text{M}\)) and lithium (0.8 \(\text{mEq/ml}\)) did not displace any of the specific radio-labeled ligand bindings in vitro.

A typical distribution of \([^{3}\text{H}]\text{-5-HT}\) binding is illustrated in the photographic atlas of coronal sections presented in Fig. 1 (Fig. 1A: total binding image, Fig. 1B: nonspecific binding image, Fig. 1C: specific binding image). All areas of the cerebral cortex, hippocampus and thalamus contained high concentrations of 5-HT receptors (Fig. 1C). The values of these specific binding densities were 1170, 2996 and 3328 fmol/mg protein for the frontal cortex, hippocampus and choroid plexus, respectively (Table 3); therefore, the densities of 5-HT receptors were extremely high in these brain areas. Long-term administration of imipramine or lithium significantly decreased specific binding activities of 5-HT receptors in the frontal cortex, hippocampus and choroid plexus (Fig. 2 and Table 3).

\([^{3}\text{H}]\text{-8-OH-DPAT}\) specific binding was enriched in the hippocampus (783 fmol/mg protein) in comparison with either the frontal cortex (365 fmol/mg protein) or the choroid plexus (36 fmol/mg protein) (Fig. 3A and Table 3). Chronic treatment with imipramine significantly decreased specific binding activities of 5-HT receptors in brain areas (Fig. 3B and Table 3). In contrast, chronic treatment with lithium significantly decreased those of 5-HT receptors in the hippocampus and choroid plexus (Fig. 3C); particularly, both drugs greatly decreased specific binding in the choroid plexus (Table 3).

\([^{125}\text{I}]\text{-Ketanserin}\) and \([^{3}\text{H}]\text{-spiperone}\) were used to label the 5-HT receptors. \([^{125}\text{I}]\text{-Ketanserin}\) specific binding was greatly enriched in the frontal cortex (621 fmol/mg protein) and striatum compared with the hippocampus (72 fmol/mg protein) or the choroid plexus (37 fmol/mg protein) (Fig. 4A and Table 3). Similarly, \([^{3}\text{H}]\text{-spiperone}\) specific binding was also remarkable in the frontal cortex (1300 fmol/mg protein) compared with the hippocampus (110 fmol/mg protein) or the choroid plexus (7 fmol/mg protein) (Table 3). Chronic treatment with imipramine or lithium abolished specific binding activities of 5-HT receptors in brain areas (Fig. 4B, 4C) and significantly decreased specific bindings of these two radio-labeled ligands in the frontal cortex, hippocampus and choroid plexus (Table 3).

**Discussion**

Initially, in order to define the pharmacological profile of 5-HT receptor subtypes by quantitative receptor autoradiography, the affinity for displacers of radio-labeled ligands and the specific binding of radio-labeled ligands were compared in three brain areas: frontal cortex, hippocampus and choroid plexus. The rank order for affinity or density of 5-HT receptors was choroid plexus > hippocampus > frontal cortex (Tables 2 and 3). Our data were in close agreement with the findings of Pazos and Palacios (7). In regard to the different subtypes of 5-HT receptors, the rank order for 5-HT receptors was choroid plexus > hippocampus > frontal cortex (Tables 2 and 3). In this way,
Fig. 1. Autoradiographic localization of $[^3H]$-5-HT binding sites in the rat brain. Autoradiograms show the intense labeling of the total binding (A), non-specific binding (B) and specific binding (C). Anatomical level in coronal sections=A 2.8 mm (2.8 mm anteriorly from the frontal zero plane). C.C: Cerebral cortex, Hip: Hippocampus, Tha: Thalamus. Bar=0.3 cm.

Fig. 2. Autoradiograms of specific $[^3H]$-5-HT binding to the coronal sections (A 2.6 mm) at the level of the hippocampus of rats treated with imipramine (A) and lithium (B) for 21 days. The specific binding images were obtained by subtracting the nonspecific binding images from the total binding images. C.C: Cerebral cortex, Hip: Hippocampus. Bar=0.3 cm.
Fig. 3. Autoradiograms of specific [3H]-8-OH-DPAT binding to the coronal sections (A 2.8 mm) at the level of the hippocampus of rats treated with saline (A), imipramine (B) and lithium (C) for 21 days. C.C: Cerebral cortex, Hip: Hippocampus. Bar=0.3 cm.

Fig. 4. Autoradiograms of specific [125I]-CYP binding to the sagittal brain sections from rats treated with saline (A), imipramine (B) and lithium (C) for 21 days. Anatomical level in sagittal sections = L 1.0–1.3 mm (1.0–1.3 mm laterally from the median plane). F.C: Frontal cortex, G.P: Globus pallidus, Hip: Hippocampus, S.N: Substantia nigra. Bar=0.3 cm.
Fig. 5. Autoradiograms of specific [3H]-mesulergine binding to the sagittal brain sections (L 0.7–1.3 mm) from rats treated with saline (A), imipramine (B) and lithium (C) for 21 days. C.P: Choroid plexus, F.C: Frontal cortex, Hip: Hippocampus. Bar=0.3 cm.

Fig. 6. Autoradiograms of specific [125I]-ketanserin binding to the sagittal brain sections (L 2.0–2.2 mm) from rats treated with saline (A), imipramine (B) and lithium (C) for 21 days. F.C: Frontal cortex, Hip: Hippocampus, Str: Striatum. Bar=0.3 cm.
5-HT1 receptors showed a very heterogeneous distribution in the rat brain. The rank order for [125I]-ketanserin labeled 5-HT2 receptors was frontal cortex > hippocampus > choroid plexus. There were two types of specific [125I]-ketanserin binding sites: one with high affinity (K1) and the other with low affinity (K2). Likewise, both high and low affinities of [3H]-ketanserin have been presented in binding studies (21) and in autoradiographic studies (8). The rank order for [3H]-spiperone labeled 5-HT2 receptors was frontal cortex/hippocampus > choroid plexus. In this way, the concentrations of 5-HT2 receptors were very high in the frontal cortex, but very low in the hippocampus and choroid plexus. It can be stated that the contents of 5-HT2 receptors are much higher in telencephalic areas than in meso- or metencephalic areas (8). The three brain areas investigated in this study have been found to contain nerve-endings from the serotoninergic neurons which are mainly located in the raphe nuclei. It has been ascertained that the pharmacological characterization of 5-HT binding to some brain areas obtained by autoradiographic studies correlates well with that reported in membrane-binding studies (7, 8).

Secondly, the effects of long-term administration of imipramine or lithium on different 5-HT receptor subtypes were studied in these three brain areas. Imipramine decreased the densities of 5-HT1, 5-HT1A, 5-HT1C and 5-HT2 sites in the frontal cortex, hippocampus and choroid plexus. These results on 5-HT1, 5-HT1A and 5-HT1C sites obtained by autoradiographic studies using only a single dose of radiolabeled ligands agree with our data reported in previous binding studies (14). Furthermore, the present study newly revealed that imipramine decreases the density of 5-HT1C sites in the frontal cortex, hippocampus and choroid plexus, and also that of 5-HT2 receptors in the choroid plexus. Although imipramine had an inclination to decrease the densities of 5-HT1C and 5-HT2 sites in whole brain areas, in particular, the decrease of 5-HT1C sites in the choroid plexus was extreme. From our data, the inhibition of 5-HT1A sites induced by imipramine may directly change the 5-HT-stimulated adenylate cyclase activity in the synaptic membranes because 5-HT1A sites negatively link to adenylate cyclase and possibly the GTP binding proteins (G1 and/or G0) in the hippocampus (2, 22). On the other hand, the inhibition of 5-HT1C sites may suggest a reduction of the 5-HT-stimulated phosphoinositide (PI) turnover linked to this receptor subtype (23). 5-HT2 receptors also positively link to PI turnover in the cerebral cortex, and 5-HT-stimulated PI hydrolysis may be mediated by the postsynaptic 5-HT2 receptors in these areas (24). Therefore, the inhibition of 5-HT2 receptors induced by imipramine may reduce 5-HT-stimulated PI turnover.

Lithium decreased the densities of 5-HT1, 5-HT1C and 5-HT2 sites in the frontal cortex and those of 5-HT1, 5-HT1A 5-HT1C and 5-HT2 sites in the hippocampus and choroid plexus. With the exception of 5-HT1C sites, these results obtained from the present study are in agreement with the findings of previous reports (14-16). The present study newly revealed that lithium decreases the density of 5-HT1C sites in the frontal cortex, hippocampus and choroid plexus, although these results were the same as those obtained with imipramine. The lithium-induced reduction of density of 5-HT1 receptors in the frontal cortex was due to the 5-HT1A sites themselves. From the result that lithium caused the inhibition of 5-HT1A sites in the hippocampus, lithium as well as imipramine may take part in the 5-HT-stimulated adenylate cyclase activity in the synaptic membranes. In the frontal cortex, lithium, unlike imipramine did not affect the 5-HT1A sites. This difference between lithium and imipramine may explain their different efficacies in treating manic-depressive illness and depressive illness, as previously discussed (14). The inhibitions of 5-HT1C and 5-HT2 sites induced by lithium in whole brain areas, as well as imipramine, may reduce the 5-HT-stimulated PI turnover linked to these receptor subtypes. Recent work showed that acute and chronic lithium treatments reduce the PI response to different receptor agonists in rat cerebral cortex (25). Most recently, it has been reported that lithium blocks both adrenergic and cholinergic agonist-induced increases in the GTP binding to rat cerebral cortical membranes; therefore, these findings suggest GTP binding proteins.
(Gs and Gi or Go) are the molecular sites of action for both the antimanic and antidepressant effects of lithium (26). Further investigation of the action of imipramine on the GTP binding proteins is necessary to elucidate the difference between lithium and imipramine. Moreover, in rat hippocampal slices, it has been suggested that lithium diminishes both the cholinergic agonist-induced activation of protein kinase C (PKC) by diacylglycerol (DG) and the PI hydrolysis by phospholipase C (27). Although the effect of lithium on 5-HT-activated PKC is still unknown, our results that lithium caused inhibitions of 5-HT1C and 5-HT2 sites may suggest that lithium can diminish 5-HT-activation of PKC by DG. In addition to this, further work is necessary to clarify whether imipramine follows the same mechanism as lithium or not.

In conclusion, sites with the pharmacological properties of 5-HT receptors are heterogeneously distributed in the rat brain. The hippocampus was enriched in 5-HT1A and 5-HT1B sites; the choroid plexus contained abundant 5-HT1C sites; and the frontal cortex was enriched in 5-HT2 receptors. Chronic imipramine administration decreased the densities of 5-HT1, 5-HT1A, 5-HT1C and 5-HT2 sites in these three areas. Chronic lithium administration decreased the densities of 5-HT1, 5-HT1C and 5-HT2 sites in the frontal cortex and those of 5-HT1, 5-HT1A, 5-HT1C and 5-HT2 sites in the hippocampus and choroid plexus. These results obtained by the autoradiographic technique seem to indicate that this is a good approach for studying the local effects of these drugs.

Acknowledgments: This study was supported by Grant (63-A) from The National Center of Neurology and Psychiatry (NCNP) of the Ministry of Health and Welfare, Japan. We would like to thank Amersham Japan Co., Ltd. for their help in the autoradiographic analysis of 5-HT receptors and thank Miss Chika Imai for excellent technical assistance.

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