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Synthesis and Turnover of ^{3}H-5-Hydroxytryptamine in the Lateral Cerebroventricle[1,2]

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KANTAK, K. M., M. J. WAYNER, H. A. TILSON, L. P. DWOSKIN AND J. M. STEIN. *Synthesis and turnover of ^{3}H-5-hydroxytryptamine in the lateral cerebroventricle.* PHARMAC. BIOCHEM. BEHAV. 8(2) 153–161, 1978. – The lateral cerebroventricle was perfused using two different labelling procedures in three separate experiments on 5-hydroxytryptamine metabolism. The sensitivity of 5-hydroxytryptamine metabolism to various drugs was subsequently determined. The results demonstrate that two serotonin reuptake blockers, imipramine and fluoxetine, increase the efflux of ^{3}H-5-hydroxytryptamine into the ventricle without affecting the efflux of ^{3}H-5-hydroxyindoleacetic acid. BL-3912 A, a drug with weak serotonin agonist activity, also increased the efflux of ^{3}H-5-hydroxytryptamine into the ventricle.

Tryptophan 5-Hydroxytryptamine 5-Hydroxyindoleacetic acid Lateral cerebroventricle Imipramine Fluoxetine BL-3912A Dimoxamine

HISTOCHEMICAL fluorescence evidence [9] indicates that imipramine blocks the reuptake of 5-hydroxytryptamine (5-HT) in the central nervous system. On the basis of these data, the effects of imipramine on the synthesis and turnover of 5-HT have been investigated [3,7]. Brain levels of 5-HT either remain unchanged or increase after imipramine treatment. Brain levels of 5-hydroxyindoleacetic acid (5-HIAA) decrease after imipramine administration. Imipramine reduces the discharge rate of raphe neurons [1, 2, 11]. This effect is related to negative feedback produced by increased extraneuronal 5-HT following imipramine treatment and is consistent with the hypothesis that imipramine blocks the reuptake of 5-HT. Tritiated tryptophan has been used to study the effects of imipramine on the synthesis of ^{3}H-5-HT. Imipramine increased whole brain ^{3}H-5-HT [10]. Tritiated 5-HIAA was not determined in this study. Perfusing the lateral cerebroventricles after ^{3}H-L-tryptophan infusion revealed an increase in ^{3}H-5-HT efflux after treatment with chlorimipramine [8]. Determinations of ^{3}H-5-HIAA were not made. However, there was a simultaneous inhibition of raphe firing and increased efflux of ^{3}H-5-HT after chlorimipramine treatment.

The purpose of the present investigation was to measure the effect of several drugs on 5-HT metabolism in terms of the efflux of ^{3}H-5-HT and ^{3}H-5-HIAA into the lateral cerebroventricle. Two different labelling procedures were utilized. One experiment involved perfusing with ^{3}H-L-tryptophan and determining the subsequent changes in ^{3}H-5-HT and ^{3}H-5-HIAA effluxes following peripheral imipramine administration. Another experiment involved pulse-labelling ^{3}H-5-HT into the lateral cerebroventricle and perfusing with 0.9% bacteriostatic saline. The changes in ^{3}H-5-HT and ^{3}H-5-HIAA effluxes following peripheral imipramine administration were measured. In the final experiment, ^{3}H-L-tryptophan was pulse-labelled into the lateral cerebroventricle prior to perfusion with 0.9% bacteriostatic saline. The changes in ^{3}H-5-HT and ^{3}H-5-HIAA effluxes following peripheral administration of imipramine, fluoxetine and BL-3912 A were measured. Fluoxetine is a drug which is more potent than imipramine in inhibiting the reuptake of 5-HT [13]. BL-3912 A has been shown to increase the steady-state levels of brain 5-HT and 5-HIAA, as well as the rate of 5-HT turnover in the neocortex [4].

EXPERIMENT 1

PERFUSION OF ^{3}H-L-TRYPTOPHAN INTO THE LATERAL CEREBROVENTRICLE

The purpose of Experiment 1 was to determine the effects of imipramine hydrochloride on the synthesis and turnover of 5-HT. Rats were perfused with ^{3}H-L-tryptophan in the lateral cerebroventricle. Following a baseline period, animals were injected with either imipramine hydrochloride, 5 or 15 mg/kg, saline, or were not given an injection.

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METHOD

Animals

One male and two female hooded rats (301–367 g) from our colony were used in this experiment. Animals were housed in individual living cages. They had free access to Purina lab chow blocks and water. Animals were kept on a constant light/dark cycle. The 12 hr light phase began at 0600 hr and was followed by a 12 hr dark phase. The room temperature was maintained at 21° ± 1°C.

Surgery and Histology

Surgery was performed under Equi-Thesin anaesthesia (Jensin-Salsbery Laboratories) at a dose of 3 cc/kg. Each animal was implanted with a concentric push-pull cannula in the right lateral cerebroventricle according to predetermined DeGroot [6] coordinates: AP −5.4, L−2.0 and V −8.1 mm from the interaural line. The inner cannula extended 0.25 to 0.50 mm beyond the end of the outer cannula. Four stainless steel screws were used to attach the cannula to the skull and the implant was secured with acrylic dental cement. There was at least one week of post-operative care prior to the start of the experiment.

When the experimental conditions were terminated all animals were perfused intracardically, first with 0.9% NaCl, and then with neutralized 10% Formalin plus 0.9% NaCl. The brains were removed, frozen, and sectioned at 60 μ. Tissue was stained with cresyl violet and examined to determine the location of the cannula tip.

Apparatus

The test chamber consisted of a 20 × 20 × 20 cm Plexiglas box with a standard stainless steel rod grid floor. All push-pull cannulae perfusions were performed with a LKB Model 4912 A tubing pump. All radioactive determinations were made with a Tracerlab Corumatic 100 A scintillation counter.

Drugs

Imipramine hydrochloride, 5 and 15 mg, was dissolved in 1 ml of isotonic NaCl. Isotonic NaCl was used for the vehicle control. All injection volumes were 1 ml/kg and were injected intraperitoneally.

Procedure

Animals were perfused for 75 min at an average rate of 37.0 ± 0.5 μl/min. The perfusion medium contained 5 μCi ^{3}H–L–tryptophan (specific activity = 2.7 or 7.9 Ci/mmole, New England Nuclear) per 5 ml of artificial cerebrospinal fluid (ACSF). Fifteen 5 min samples of perfusate were collected in vials containing 0.1 ml of 0.1 N HCl. Forty min after the start of the perfusion, animals were subjected to one of four experimental treatments: no injection control, isotonic NaCl control, imipramine hydrochloride 5 mg/kg or 15 mg/kg. All animals received all 4 treatments in a random order and at least 48 hr separated each perfusion.

A 20 μl aliquot from each 5 min sample was pipetted into a glass scintillation counting vial (Kimble Products) containing 5 drops of Bio-Solv (Beckman Instruments) and 10 ml of a scintillation cocktail (6 g PPO/1 toluene). The vials were then placed in the scintillation counter. The cpm/20 μl were corrected for background (30.7 ± 1.1 cpm), efficiency (41.9% ± 0.3%), and dilution with the HCl (64.78% ± 0.4%). Data for the aliquots were calculated to dpm/20 μl.

Samples taken 15–25 min prior to injection and 10–35 min following the injection were further analyzed by thin layer chromatography (TLC) for bioconversion of ^{3}H–L–tryptophan to ^{3}H–5–HT and ^{3}H–5–HIAA. For each sample analyzed, 20 μl of perfusate was spotted on individual cellulose coated TLC plates (Brinkman). In addition 0.5 μl (5 μg dissolved in 0.1 N HCl) of the following nonlabelled carrier standards was spotted on each plate: L–tryptophan (Calbiochem), L–5–hydroxytryptophan ethyl ester (Calbiochem), 5–hydroxytryptamine creatinine sulfate (Calbiochem), and 5–hydroxyindoleacetic acid cyclohexylammonium salt (Calbiochem). A ^{3}H–L–tryptophan standard plate was prepared in the same manner as above for each perfusion. However, 15 μl of ACSF and 5 μl of a freshly prepared solution (1 μCi ^{3}H–L–tryptophan per 0.5 ml ACSF) were spotted on this TLC plate. The TLC plates were developed in a bidirectional solvent system. Solvent 1 consisted of butanol, 1.0 N formic acid, and methanol (3:1:1). Solvent 2 consisted of isopropanol, ammonia, and triple distilled water (8:1:1). Upon removal from the second solvent the four spots on each plate were detected with a 0.4% solution of ninhydrin plus acetone and with diazotized m-nitroanaline. Each spot and the origin was cut into two, 1 × 2 cm strips, and placed into a counting vial containing 1.0 ml methanol. The strips in the methanol were allowed to elude for 20 hr before the addition of the scintillation cocktail. The vials were then placed in the scintillation counter. The cpm/20 μl were corrected for background (26.3 ± 0.4 cpm), efficiency (30.9% ± 0.4%), dilution with the HCl (64.78% ± 0.4%), and recovery of counts from the corresponding aliquot vial (61.1% ± 0.04%). The final dpm were converted to nCi of metabolite formed per μCi of tryptophan perfused in each 5 min sample.

In a separate study, the Rf values of several other indoles were determined. Five μl (5 μg dissolved in 0.1 N HCl) of tryptamine (Regis), 5–methoxytryptamine (Calbiochem), 5–hydroxytryptophol (Regis), 5–methoxytryptophol and 5–methoxy-indole-3-acetic acid (Regis) were spotted and developed using the bidirectional chromatography procedures described above. The spots were detected with Erlich's reagent (7% v/v).

RESULTS

Microscopic examination of the brain tissue revealed one animal's cannula tip in the lateral cerebroventricle between AP 5.0 to 4.6 and another animal's cannula tip in the ependymal layer of the lateral cerebroventricle between AP 6.6 to 6.2. The tip of the third animal's cannula was situated in the corpus callosum between AP 5.4 to 5.0 but was less than 0.5 mm above the ventricle. Therefore, the inner cannula protruded into the ventricle. Data are reported on all three animals.

Aliquot Analysis

Data collected from the eight preinjection samples and the seven postinjection samples were analyzed by means of a 3 × 15 analysis of variance with repeated measures [12]. Because there were no significant differences, data from the no injection control and the isotonic NaCl control were combined to form a single control value for each 5 min

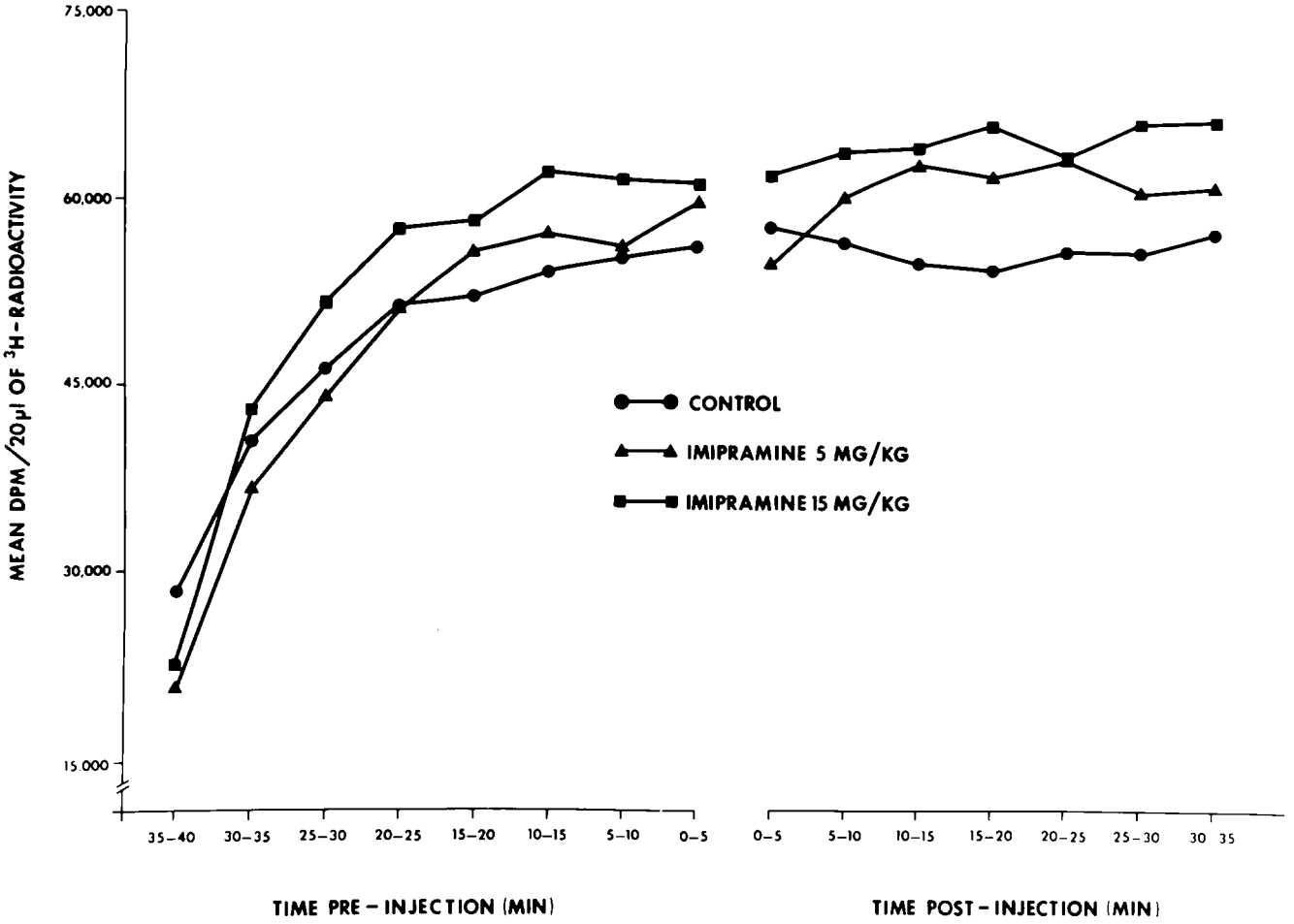

FIG. 1. Mean dpm/20 μl of ^{3}H-radioactivity found in samples taken 0–40 min prior to injection and 0–35 min following the injection. Control ●——●; Imipramine hydrochloride 5 mg/kg ▲——▲; Imipramine hydrochloride 15 mg/kg ■——■.

TABLE 1

DISTRIBUTION OF REFERENCE STANDARDS AND ^{3}H FROM ^{3}H-L-TRYPTOPHAN ON THE TLC PLATE AFTER BIDIRECTIONAL TLC SEPARATION

Reference Standards	RF Value		Mean % of Total dpm on ^{3}H-L Tryptophan Standard Plate ± SEM
	Solvent 1	Solvent 2	
L-Tryptophan	0.61 ± 0.004	0.35 ± 0.005	97.9 ± 0.1
5-Hydroxytryptophan	0.72 ± 0.007	0.88 ± 0.006	0.3 ± 0.1
5-Hydroxytryptamine	0.50 ± 0.005	0.65 ± 0.012	0.3 ± 0.1
5-Hydroxyindoleacetic acid	0.76 ± 0.004	0.24 ± 0.007	0.3 ± 0.1
Origin	—	—	1.2 ± 0.1
Tryptamine	0.72	0.75	—
5-Hydroxytryptophol	0.85	0.75	—
5-Methoxytryptamine	0.65	0.85	—
5-Methoxytryptophol	0.92	0.43	—
5-Methoxyindoleacetic acid	0.78	0.46	—

sample. The factors were the 3 treatments and the fifteen 5 min time periods pre- and postinjection. The dpm/20 μl in the pre- and postinjection samples for the drug and control treatments are presented in Fig. 1. There were no significant differences among the dpm/20 μl for the drug treatments and the control in either the pre- or postinjection samples. As would be expected, there was a significant main effect in the dpm/20 μl for the time factor, $F(14,28) = 14.85$, $p<0.01$. A Tukey A test performed among the dpm/20 μl of samples taken 15–20 and 20–25 min prior to injection and 10–15, 15–20, 20–25, 25–30, 30–35 min following the injection indicated no

TABLE 2

ABSOLUTE AMOUNTS OF 5-HYDROXYTRYPTAMINE (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) FORMED IN TWO SAMPLES PRIOR TO DRUG ADMINISTRATION. DATA ARE THE MEAN ± SEM. N CI METABOLITES FORMED PER μ CI OF TOTAL ^{3}H-RADIOACTIVITY IN TWO SAMPLES TAKEN 15–20 AND 20–25 MIN PRIOR TO INJECTION

Treatment	5-HT	5-HIAA
Control	8.9 ± 1.5	12.5 ± 5.8
Imipramine 5 mg/kg	8.5 ± 3.7	4.8 ± 3.4
Imipramine 15 mg/kg	9.0 ± 2.6	6.7 ± 2.6

significant differences. Therefore the dpm/20 μl were similar in all pre- and postinjection samples upon which the TLC analyses were performed.

TLC Analysis

Examination of the standard plates revealed good specificity for the TLC separations. The majority of the radioactivity on the standard plates was detected at the authentic L–tryptophan spot. A small percentage of the radioactivity was nonspecific and appeared at the spots of the compounds cochromatographed with ^{3}H–L–tryptophan or remained at the origin. These data are presented in Table 1 along with the Rf values of the compounds cochromatographed with the ^{3}H–L–tryptophan and the Rf values of the compounds determined without concurrent application of ^{3}H–L–tryptophan.

There were no significant differences between the control injection and no injection control for 5–HT and 5–HIAA. Data for the two treatments were combined to form a single control value. The absolute nCi amounts of 5–HT and 5–HIAA formed in samples taken 15–25 min prior to drug administration per μCi of total ^{3}H are presented in Table 2. A one-way analysis of variance with repeated measures was performed [12] comparing the preinjection amounts of 5–HT or 5–HIAA found in the control perfusion to each drug perfusion. These tests revealed no significant differences in the preinjection amounts of 5–HIAA or 5–HT among the three treatments.

Data from samples taken 10–35 min postinjection, expressed as the percent change from the preinjection 5–HT and 5–HIAA amounts for each treatment, are presented in Fig. 2. A one-way analysis of variance with repeated measures was performed [12] comparing the percent changes in the control and drug perfusion for 5–HT or 5–HIAA. For the 10–35 min postinjection period there were significant differences in 5–HT, $F(2,4) = 37.98$, $p<0.01$. There were no significant differences in 5–HIAA. These results confirm that imipramine increases 5–HT availability by decreasing reuptake and subsequent deamination.

EXPERIMENT 2

INFUSION OF ^{3}H–5–HT IN THE LATERAL CEREBROVENTRICLE

The purpose of Experiment 2 was to determine the effect of imipramine hydrochloride on 5–HT turnover. Rats were infused with ^{3}H–5–HT into the lateral cerebroventricle 1 hr prior to push-pull perfusion with bacteriostatic saline. Following a baseline period, animals were

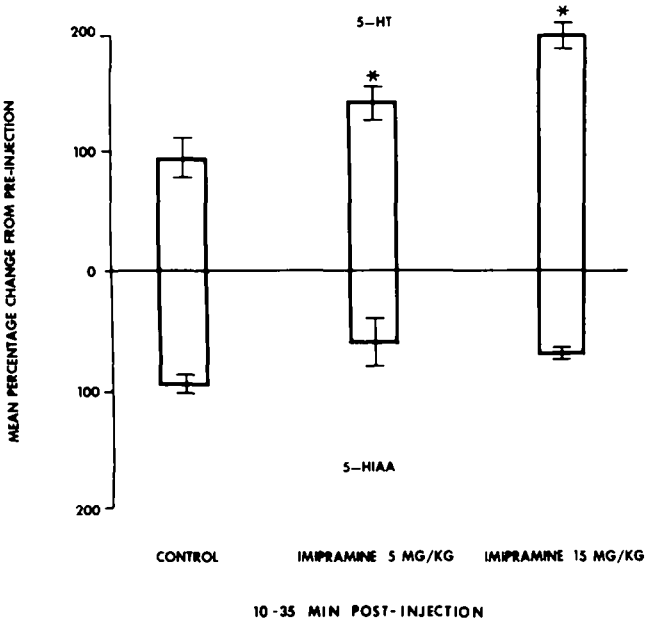

FIG. 2. Mean ± SEM percent change in nCi/μCi from preinjection 5–HT and 5–HIAA for the 10–35 min postinjection period.. *Significantly different from the control.

injected with either imipramine hydrochloride 15 mg/kg or saline.

Animals

One male and three female hooded rats (228–384 g) from our colony were used in this experiment. The surgery and histological procedures were the same as in the preceding experiment.

Apparatus

The testing chamber consisted of a 20 x 20 x 50 cm Plexiglas box with a standard stainless steel rod grid floor. All push-pull cannulae perfusions were performed with a Sage Instruments Model 375A tubing pump. All radioactive determinations were made with a Tracerlab Corumatic 100 A scintillation counter.

Drugs

Imipramine hydrochloride (15 mg) was dissolved in 1 ml of isotonic NaCl. Isotonic NaCl was used for the vehicle control. All injection volumes were 1 ml/kg and were injected intraperitoneally.

Procedure

Animals were infused, via the push-pull cannula, with 10 μCi (470 ng) of ^{3}H–5–hydroxytryptamine binoxalate (specific activity = 5.7 Ci/mmole, New England Nuclear) and 1 μl of 0.9% bacteriostatic saline 1 hr prior to push-pull perfusion. This procedure utilized a Harvard infusion pump and the liquid was infused at a rate of 1 μl/min. Animals were perfused at an average rate of 37.43 ± 0.48 μl/min for 120 min with 0.9% bacteriostatic saline. Twelve 10 min samples of perfusate were collected in vials containing 0.2 ml of 1.0 N formic acid. Sixty min after the start of the perfusion, animals were subjected to one of two experimental treatments: 0.9% NaCl control or imipramine hydro-

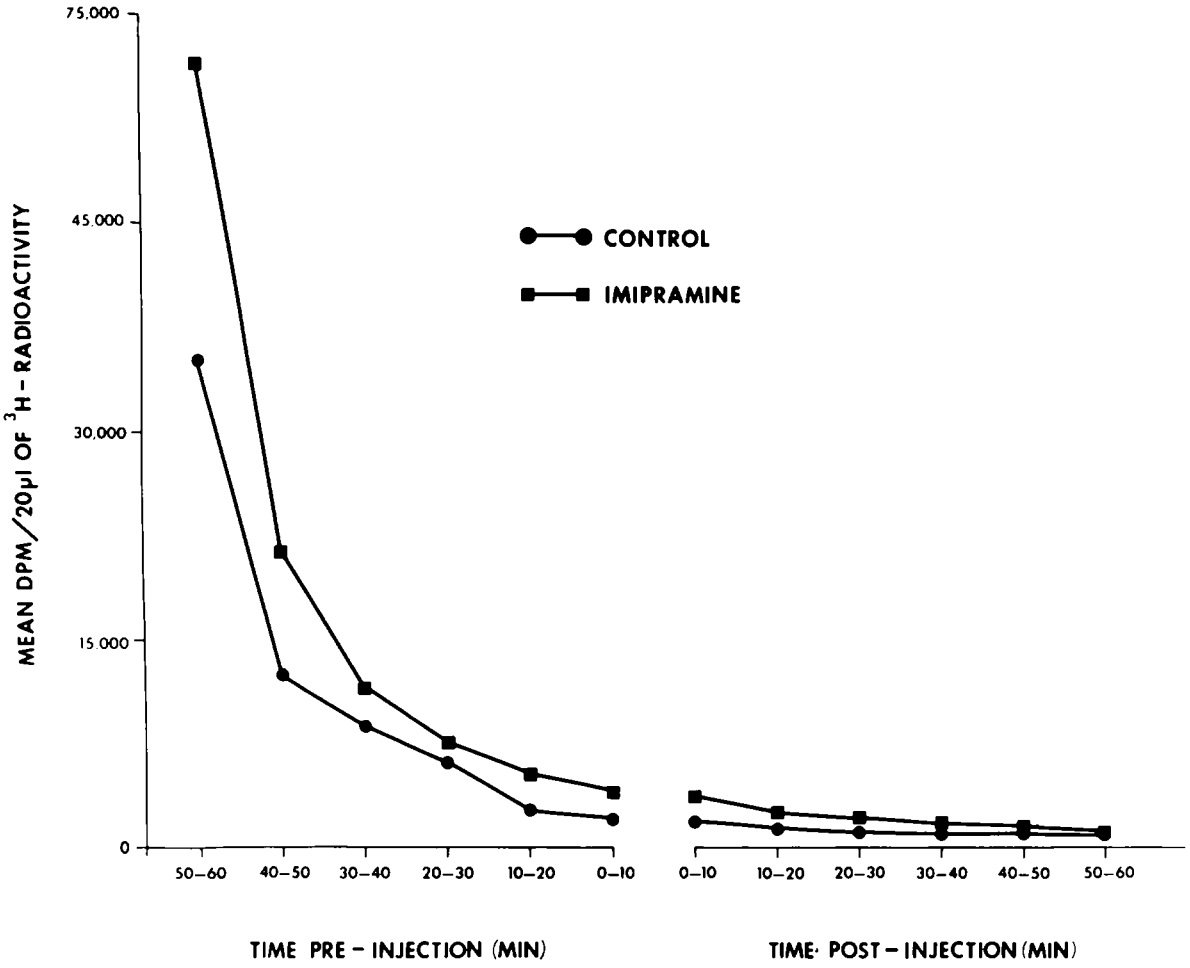

FIG. 3. Mean dpm/20 μl of ^{3}H-radioactivity found in samples taken 0–60 min prior to injection and 0–60 min following the injection. Control ●—— —●; Imipramine hydrochloride 15 mg/kg ■——■.

TABLE 3

DISTRIBUTION OF REFERENCE STANDARDS AND ^{3}H FROM ^{3}H-5-HYDRTOXYTRYPTAMINE ON THE TLC PLATE AFTER BIDIRECTIONAL TLC SEPARATION

Reference standards	RF value		Mean % of total dpm on ^{3}H-5-Hydroxytryptamine
	Solvent 1	Solvent 2	Standard Plate ± SEM
5-Hydroxytryptamine	0.52 ± 0.006	0.67 ± 0.003	97.88 ± 0.71
5-Hydroxyindoleacetic acid	0.77 ± 0.003	0.26 ± 0.002	0.89 ± 0.38
Origin	—	—	1.23 ± 0.51

chloride 15 mg/kg. All animals received both treatments in a counterbalanced order and at least 48 hr separated each perfusion. The aliquots were analyzed in the same manner as in the preceding experiment. The cpm/20 μl were corrected for background (24.1 ± 1.08 cpm), efficiency (39.17% ± 2.11%), and dilution with the formic acid (65.08% ± 0.5%). Data for the aliquots were calculated to dpm/20 μl.

Samples taken 0–20 min prior to injection and 10–60 min following the injection were further analyzed by TLC for turnover of ^{3}H-5-HT to ^{3}H-5-HIAA. The TLC plates were spotted as in the preceding experiment except that the nonlabelled carrier standards, 5-HT and 5-HIAA, were dissolved in 1.0 N formic acid. A ^{3}H-5-hydroxytryptamine standard plate was prepared for each perfusion. Twenty μl of a freshly prepared solution containing 0.9% bacteriostatic saline, formic acid, and ^{3}H-5-hydroxytryptamine were spotted on the TLC plate. There were approximtely 2000 to 4000 dpm/20 μl. The plates were developed as in the preceding experiment and the spots were detected with diazotized m-nitroanaline. Each spot and the origin were cut, eluded, and counted in the same manner. The cpm/20 μl were corrected for background (20.6 ± 0.61 cpm), efficiency (30.33% ±

0.005%), dilution with the formic acid (65.08% ± 0.5%), and recovery of counts from the corresponding aliquot vial (57.66% ± 0.04%).

RESULTS

Histology

Microscopic examination of the tissue revealed all animals' cannula tip in the lateral cerebroventricle except for one animal whose cannula tip was in the ependymal tissue just bordering the lateral cerebroventricle and less than 0.5 mm from the ventricle. The extent of the lesions ranged from AP 6.2 to 5.0. Data are reported on all four animals.

Aliquot Analysis

Data collected from the six preinjection samples and the six postinjection samples were analyzed by means of a 2 x 12 analysis of variance with repeated measures [12]. The factors were the 2 treatments and the twelve 10 min time periods pre- or postinjection. The dpm/20 μl in the pre- and postinjection samples for imipramine and control treatments are presented in Fig. 3. There were no significant differences between the dpm/20 μl for the imipramine treatment and the control in either pre- or postinjection samples. There was a significant main effect for the time factor $F(11,33) = 12.29$, $p<0.01$. A Tukey A test performed among the dpm/20 μl of the two samples taken 0–20 min prior to the injection, and the five samples taken 10–60 min following the injection, indicated no significant differences. The dpm/20 μl were similar in all pre- and postinjection samples upon which the TLC analyses were performed.

TLC Analysis

Examination of the standard plates revealed good specificity for the TLC separations. The majority of the radioactivity on the standard plates was detected at the authentic 5–HT spot. A small percentage of radioactivity was nonspecific and appeared at the spot of authentic 5–HIAA or remained at the origin. These data are presented in Table 3 along with the Rf values of 5–HT and 5–HIAA.

Data from samples taken 10–60 min postinjection, expressed as the percent change from preinjection 5–HT and 5–HIAA amounts for each treatment, revealed no significant differences in either compound following imipramine hydrochloride 15 mg/kg. The effects of imipramine might have been masked using percent change from pre-injection since in both the control and imipramine treatments a steady decline over time in the amounts of 5–HT and 5–HIAA occurred. This is probably related to the labelling technique. If the data is expressed absolutely as nCi/10–60 min postinjection, a significant increase in 5–HT, $t(3) = 2.66$, $p<0.05$, is observed following imipramine hydrochloride 15 mg/kg. No significant differences in 5–HIAA were detected. Individual *t*-tests for correlated observations were performed [12]. These data are presented in Fig. 4.

The nCi/20 min of 5–HT and 5–HIAA found in samples taken 0–20 min prior to drug administration are presented in Table 4. Individual *t*-tests for correlated observations were performed comparing the preinjection nCi/20 min found in the control perfusion to that found in the

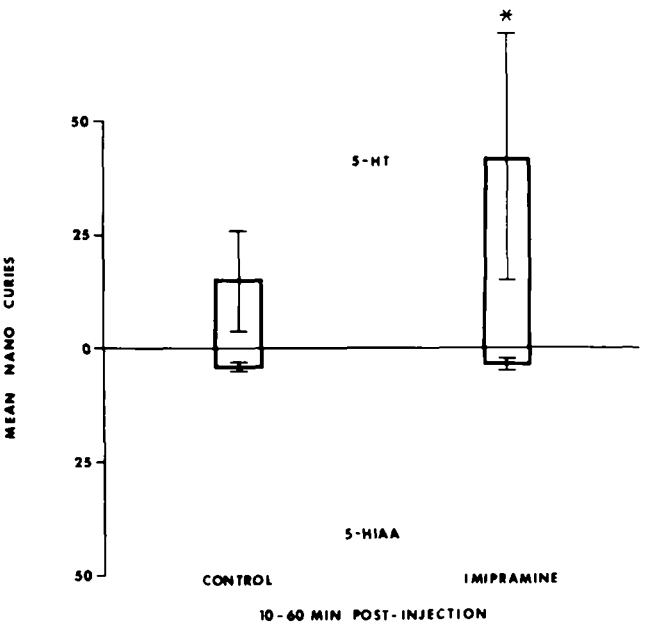

FIG. 4. Mean ± SEM nCi/10–60 min postinjection for 5–HT and 5–HIAA. *Significantly different from the control.

TABLE 4

ABSOLUTE AMOUNTS OF 5-HYDROXYTRYPTAMINE (5-HT) AND 5-HYDROXYINDOLE ACETIC ACID (5-HIAA) FOUND IN TWO SAMPLES TAKEN PRIOR TO DRUG ADMINISTRATION. DATA ARE THE MEAN ± SEM. N CI 0–10 AND 10–20 MIN PRE-INJECTION

Treatment	5-HT	5-HIAA
Control	14.39 ± 6.27	2.90 ± 1.01
Imipramine 15 mg/kg	42.64 ± 23.66	4.27 ± 0.67

imipramine perfusion. These tests revealed no significant differences in the preinjection amounts of 5–HT or 5–HIAA. Thus the differences observed postinjection are drug induced. These results confirm, as in Experiment 1, that imipramine increases 5–HT availability.

EXPERIMENT 3

INFUSION OF ^{3}H–L–TRYPTOPHAN INTO THE LATERAL CEREBROVENTRICLE

The purpose of Experiment 3 was to determine the effects of imipramine hydrochloride, fluoxetine hydrochloride, and BL–3412A on 5–HT synthesis and turnover. Rats were infused with ^{3}H–L–tryptophan into the lateral cerebroventricle 1 hr prior to push-pull perfusion with 0.9% bacteriostatic saline. Following a baseline period, animals were injected with either imipramine hydrochloride 15 mg/kg, fluoxetine hydrochloride 10 mg/kg, or BL–3912A 20 mg/kg.

Animals

Two male and one female hooded rats (328–592 g) from our colony were used in this experiment. The surgery and histological procedures were the same as used in Experiment 1.

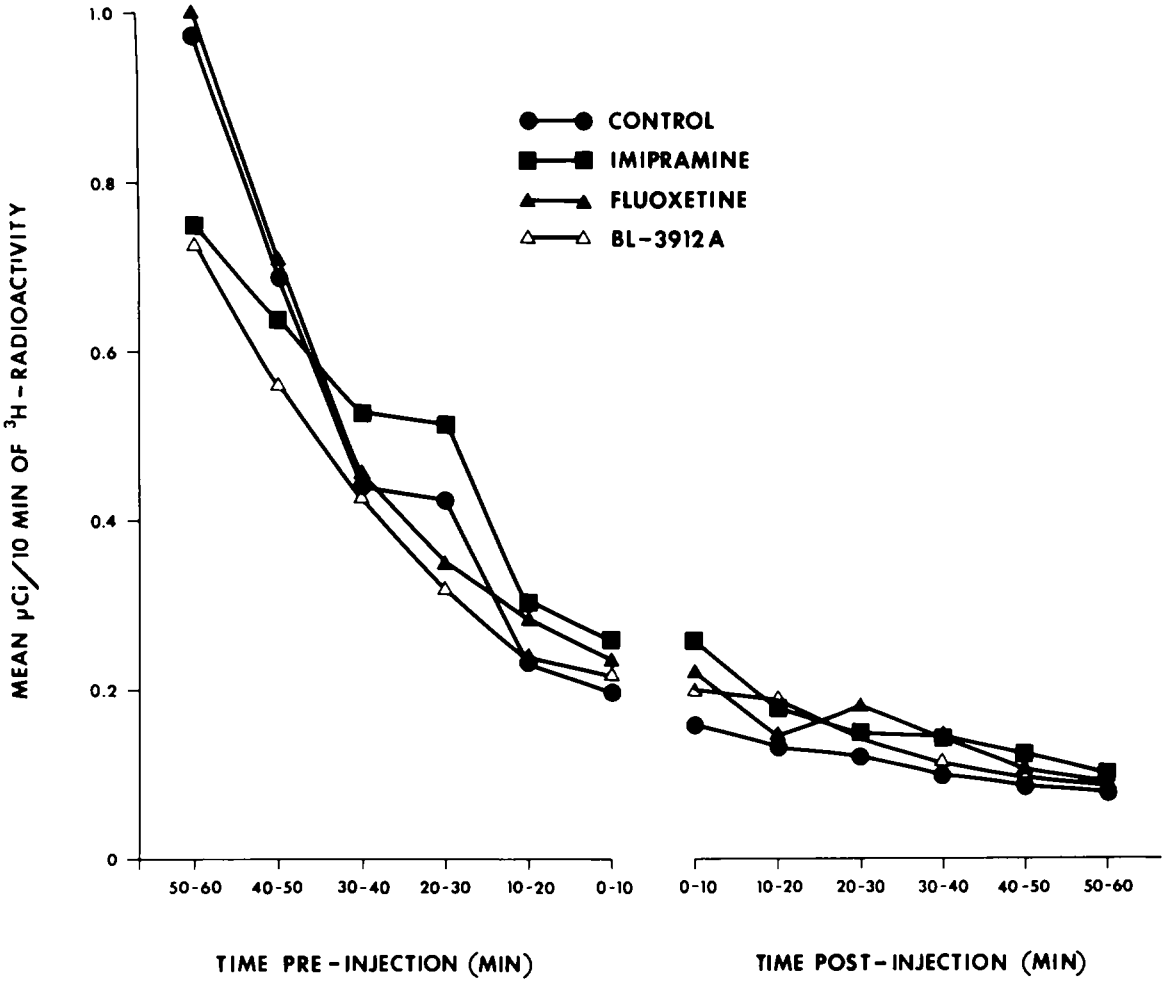

FIG. 5. Mean μCi/10 min of ^{3}H-radioactivity found in samples taken 0–60 min prior to injection and 0–60 min following the injection. Control ●——●; Imipramine hydrochloride 15 mg/kg ■——■; Fluoxetine hydrochloride 10 mg/kg ▲——▲; BL-3912A 20 mg/kg △——△.

Apparatus

The testing chamber and perfusion pump were the same as used in the preceding experiment. All radioactive determinations were made with a Beckman Model LS 100-C scintillation counter.

Drugs

Imipramine hydrochloride (15 mg), fluoxetine hydrochloride (Lilly 110140, 10 mg), and BL-3912A (dimoxamine, 20 mg) were dissolved in 1 ml of isotonic NaCl. Isotonic NaCl was used for the vehicle control. All injection volumes were 1 ml/kg and were injected intraperitonially.

Procedure

Animals were infused via the push-pull cannula, with 10 μCi (260 ng) of ^{3}H-L-tryptophan (specific activity = 7.9 Ci/mmole, New England Nuclear) and 1 μl of 0.9% bacteriostatic saline 1 hr prior to push-pull perfusion. Animals were perfused at an average rate of 19.40 ± 0.18 μl/min for 120 min with 0.9% bacteriostatic saline. Twelve 10 min samples of perfusate were collected in vials containing 0.1 ml of 1.0 N formic acid. Sixty min after the start of the perfusion, animals were subjected to one of four experimental treatments: 0.9% NaCl control, imipramine hydrochloride 15 mg/kg, fluoxetine hydrochloride 10 mg/kg, or BL-3912A 20 mg/kg. All animals received all treatments in a random order and at least 48 hr separated each perfusion. The aliquots were analyzed in the same manner as Experiments 1 and 2. The cpm/20 μl were corrected for background (24.92 ± 1.45 cpm), efficiency 39.71% ± 0.79%), and dilution with the formic acid (65.98% ± 0.22%). Data for the aliquots were calculated to μCi/10 min sample.

Samples taken 0–20 min prior to injection and 10–40 min following the injection were further analyzed by TLC as in the preceding experiment. L-tryptophan, 5-hydroxytryptophan, and 5-methoxytryptamine were added as non-labelled carrier standards. A ^{3}H-L-tryptophan standard plate was prepared as in the preceding experiment. All developing procedures were the same except that the spots were detected with Erlick's reagent 7% v/v. The cpm/20 μl were corrected for background (19.98 ± 0.22 cpm) efficiency (26.48% ± 0.79%), dilution with the formic acid (65.98% ± 0.22%), and recovery of counts from the corresponding aliquot vial (31.39% ± 2.31%).

TABLE 5

DISTRIBUTION OF REFERENCE STANDARDS AND ^{3}H FROM ^{3}H-L-TRYPTOPHAN ON THE TLC PLATE AFTER BIDIRECTIONAL TLC SEPARATION

Reference Standards	RF value		Mean % of total dpm on ^{3}H-L-tryptophan standard plate ± SEM
	Solvent 1	Solvent 2	
L-tryptophan	0.49 ± 0.02	0.37 ± 0.01	96.08 ± 1.17
5-hydroxytryptophan	0.74 ± 0.01	0.87 ± 0.01	0.83 ± 0.33
5-hydroxytryptamine	0.49 ± 0.02	0.68 ± 0.02	0.58 ± 0.19
5-hydroxyindoleacetic acid	0.77 ± 0.004	0.27 ± 0.01	0.79 ± 0.52
5-methoxytryptamine	0.62 ± 0.01	0.79 ± 0.02	1.21 ± 0.96
Origin	—	—	0.52 ± 0.29

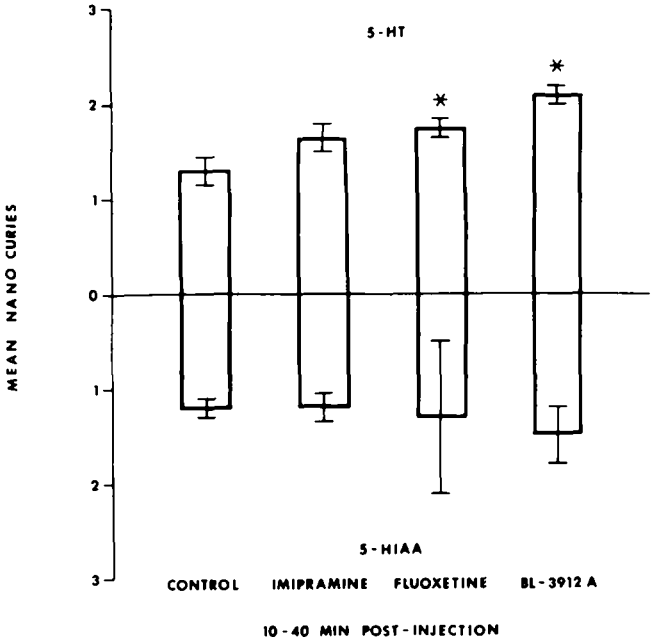

FIG. 6. Mean ± SEM nCi/10–40 min postinjection for 5–HT and 5–HIAA. *Significantly different from the control.

RESULTS

Histology

Microscopic examination of the tissue revealed all animals' cannula tip in the lateral cerebroventricle. The extents of the lesions ranged from AP 6.4 to 5.0. Data are reported on all three animals.

Aliquot Analysis

Data collected from the six 10 min preinjection samples and the six 10 min postinjection samples were analyzed by means of a 4 x 12 analysis of variance with repeated measures [12]. The factors were the 4 treatments and the twelve 10 min time periods pre- or postinjection. The μCi/10 min in the pre- and postinjection samples for control, imipramine, fluoxetine, and BL–3912A treatments are presented in Fig. 5. There were no significant differences among the treatments in either pre- or postinjection samples. There was a significant main effect for the time factor, $F(11,22) = 6.82$, $p<0.01$. A Tukey A test performed among the μCi/10 min of samples taken 0–20 min prior to the injection and 10–40 min following the injection indicated no significant differences. The μCi/10 min were similar in all pre- and postinjection samples upon which the TLC analyses were performed.

TABLE 6

ABSOLUTE AMOUNTS OF 5-HYDROXYTRYPTAMINE (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) FOUND IN TWO SAMPLES TAKEN PRIOR TO DRUG ADMINISTRATION. DATA ARE THE MEAN ± SEM. N CI 0–10 AND 10–20 MIN PRE-INJECTION

Treatment	5-HT	5-HIAA
Control	1.64 ± 0.23	1.12 ± 0.91
Imipramine 15 mg/kg	2.05 ± 0.43	1.47 ± 0.82
Fluoxetine 10 mg/kg	1.59 ± 0.31	0.79 ± 0.37
BL-3912A 20 mg/kg	2.03 ± 0.20	1.14 ± 0.40

TLC Analysis

Examination of the standard plates revealed good specificity for the TLC separations. The majority of the radioactivity on the standard plates was detected at the authentic L–tryptophan spot. A small percentage of radioactivity was non-specific and appeared at the spots of the compounds cochromatographed with the ^{3}H–L–tryptophan or remained at the origin. These data are presented in Table 5, along with the Rf values of the compounds cochromatographed with the ^{3}H–L–tryptophan.

Data from samples taken 10–40 min postinjection, expressed as the percent change from preinjection 5–HT and 5–HIAA amounts for each treatment, revealed no significant differences in either compound, following imipramine hydrochloride 15 mg/kg, fluoxetine hydrochloride 10 mg/kg, or BL–3912A 20 mg/kg. Again, as in the second experiment, a steady decline over time in the amount of 5- HT and 5–HIAA for all treatments occurred. If the data is expressed absolutely as nCi/10–40 min postinjection, a significant treatment effect is found, $F(3,6) = 9.27$, $p<0.05$, for 5–HT. Dunnett's test revealed significant increases in the nCi/10–40 min postinjection for 5–HT following fluoxetine hydrochloride 10 mg/kg ($p<0.025$) and BL–3912A 20 mg/kg ($p<0.005$). There were no significant differences between imipramine hydrochloride and the control for 5–HT ($0.05<p<0.1$). No significant differences in 5–HIAA were detected following all treatments. One

way analyses of variance for repeated measures were performed [12]. These data are presented in Fig. 6.

The nCi/20 min of 5-HT and 5-HIAA found in samples taken 0-20 min prior to drug administration are presented in Table 6. One way analyses of variance for repeated measures were performed. These tests revealed no significant differences in the preinjection amounts of 5-HT or 5-HIAA for any treatments. Thus, the differences observed postinjection were drug induced. These data confirm that fluoxetine increases 5-HT availability by decreasing reuptake and subsequent deamination. In addition, these data also confirm the increase in the steady state level of 5-HT following BL-3912A.

DISCUSSION

The results from the preceding experiments demonstrate that metabolism of radiolabelled neurochemicals in the lateral cerebroventricle during push-pull perfusion is sensitive to drug induced changes. Using two different labelling procedures, increases in ^{3}H-5-HT were detected in the lateral cerebroventricle effluent following imipramine, fluoxetine, and BL-3912A administrations. These data support a decreased 5-HT reuptake following imipramine and fluoxetine [9,13]. In addition, these results support an increased level of 5-HT in the neocortex following BL-3912A [4].

The reason for the lack of effect of these treatments on 5-HIAA is not entirely clear. The lateral cerebroventricles of rat do contain a choroid plexus which actively transports 5-HIAA from the CSF [5]. Because the ^{3}H-labelled compounds were introduced into the ventricle, the ^{3}H-5-HIAA formed might have been readily transported away under all conditions. Therefore any drug induced changes might be obscured. Such an explanation is supported further by other data collected in our laboratory (Kantak, *et al.*, manuscript in preparation) in which imipramine was shown to significantly decrease ^{3}H-5-HIAA formed from ^{3}H-5-HT perfused in solid brain tissue.

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