AUTORADIOGRAPHIC STUDIES ON DISTRIBUTION OF
L-3,4-DIHYDROXYPHENYLALANINE (L-DOPA)-\(^{14}\)C AND
L-5-HYDROXYTRYPTOPHAN (L-5-HTP)-\(^{14}\)C IN THE CAT BRAIN

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Abstract—The distribution and metabolism of L-DOPA-\(^{14}\)C and L-5-HTP-\(^{14}\)C in the cat brain were examined by means of autoradiography and chromatography. The results revealed that an appreciable amount of radioactivity in the gray matter, but not the white, and that the localization profiles of radioactivity of L-DOPA and L-5-HTP significantly differed. After L-DOPA-\(^{14}\)C administration, a high accumulation was found in the caudate nucleus, putamen and pallidum. With L-5-HTP-\(^{14}\)C administration, high radioactivity was observed in the hypothalamus, raphe nucleus, substantia nigra, inferior olivary and caudate nucleus. An analysis of the main metabolites of both amino acids in various regions of the brain was also made. When L-DOPA was given, a high concentration of dopamine was detected in the caudate nucleus, followed by the hypothalamus. In the case of L-5-HTP, a high concentration of serotonin was detected in the hypothalamus and the medulla oblongata. These results suggest that amines derived from exogenously administered L-DOPA and L-5-HTP accumulate in the brain regions known as the corresponding amine rich regions, under physiological conditions.

Deficiencies of biogenic amines in the brain are reportedly closely associated with various symptoms of Parkinsonism (1) and mental depression (2). During the last decade, L-DOPA, as a precursor of dopamine, has been widely prescribed for treating Parkinsonism and L-5-HTP has also been given to patients with symptoms of depression (3–5).

Extensive studies on the distribution and metabolism of these amino acid in rats have been carried out in our laboratory with special reference to transfer of corresponding amines into the central nervous system (CNS) (5–10). However, because of the limited information of regional anatomy of the rat brain, the regional distribution of metabolites derived from these amino acids was not elucidated.

In the present investigation, cats were used to obtain more detailed information on the distribution of radioactivity from L-DOPA-\(^{14}\)C and L-5-HTP-\(^{14}\)C and on the contents of metabolites in various regions of the brain. In experiments on L-DOPA-\(^{14}\)C uptake, MK-486, a well established inhibitor of L-aromatic amino acid decarboxylase in the peripheral tissues, was used to enhance the CNS uptake of this amino acid (11, 12). MK-486 pretreatment was not carried out in the L-5-HTP experiment, because this amino acid was decarboxylated to a lesser extent with the peripheral L-aromatic amino acid decarboxylase than with L-DOPA (13) and was taken up extensively in the brain (10).

Thus, it was demonstrated that significant radioactivities of L-DOPA and L-5-HTP
were retained in specific regions such as the caudate nucleus and hypothalamus, in the forms of the corresponding amines.

**MATERIALS AND METHODS**

*Materials:* L-DOPA-2-14C (specific activity, 114.2 µCi/mg) and L-5-HTP-3-14C (specific activity, 28.22 µCi/mg) were obtained from the Radiochemical Centre, Amersham, England and the New England Nuclear Corporation, Boston, U.S.A., respectively. The radiochemical purity of both compounds was ascertained, by the thin layer chromatography, to be over 98%. MK-486 (Carbidopa, L-2-hydrazino-α-methyl-β-(3,4-dihydroxyphenyl)-propionic acid) was provided by Merck & Co., Inc. (U.S.A.). Other various compounds used in this study were of the purest grade and were obtained from commercial sources.

*Animals and administration:* Cats weighing about 1 kg were used. For 16 hr prior to administration of the labeled compounds, food was withheld but water was given *ad libitum.* L-DOPA-2-14C diluted with the non-radioactive compound, was dissolved in physiological saline to 1 mg/ml, and administered orally in the dose of 10 mg/kg (100 µCi/body). At 30 min before administration of L-DOPA-14C, the cats were given an MK-486 dissolved in the saline (10 mg/ml) at the dose of 10 mg/kg, i.p.

L-5-HTP-14C was diluted with the non-radioactive compound to 14.3 µCi/mg and dissolved in physiological saline at the concentration of 1 mg/ml. The solution was injected into the femoral vein in a dose of 5 mg/kg (80 µCi/body).

*Autoradiography:* One and 6 hr after administration of L-DOPA-14C, cats were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and immediately exsanguinated from the carotid artery. Cats given L-5-HTP-14C were also sacrificed in the same way at 30 min and 3 hr after dosing. The brain was rapidly removed and immersed into a mixture of hexane and solid carbon dioxide. Each frozen brain was embedded in carboxyl-cellulose paste and fixed on the microtome stage. Cross sections of 50 µ thickness were serially cut with a heavy microtome (YAMATO 1111) in a room at −15°C, and freeze-dried at −15°C for 16 hr. The dried sections were allowed to contact industrial X-ray film (SAKURA Type N, Konishiroku photo. Ind., Tokyo) and exposed for 3 weeks in the film cassette.

*Analysis of radioactive materials from the various regions of the brain:* At 1 hr after L-DOPA-14C, and 3 hr after L-5-HTP-14C administration, respectively, cats were sacrificed and the whole brain was rapidly removed and dissected into nine regions, in the cold room, i.e., cerebral cortex, cerebellum, caudate nucleus, hippocampus, thalamus, amygdala, hypothalamus, pons and midbrain, and medulla oblongata. After weighing, each specimen was homogenized with 4 volumes of ice-cold 4% HClO4 (v/w) with Potter Elvehjem’s glass homogenizer and centrifuged for 20 min with 8,000 x g at 4°C. After removing the supernatant, the residue was rehomogenized with 4 volumes of 4% HClO4, using a Polytron® homogenizer (Kinematica Co., Switzerland). After centrifugation, the supernatants were combined, and pH of the solution was adjusted to 5.0–5.5 with addition of 30% KOH, and allowed to stand at 0°C overnight. After filtration, the resulting solution was lyophilized
and the residue was dissolved in a small volume of $10^{-8} \text{ N HCl}$ in 50\% ethanol.

For analysis of L-DOPA metabolites, two dimensional separation was performed with paper partition chromatography as the first direction and paper electrophoresis as the second (9). Aliquots of the brain extracts were spotted on the central zone of the filter paper (Whatman No. 50). Development was carried out with solvent system; n-butanol: gracial acetic acid : distilled water (4 : 1 : 1). After drying with cold air, the developed filter paper was again subjected to electrophoresis to the transverse direction in 25 mM phosphate buffer of pH 6.5, applying 400 V for 1.5 hr. After drying, the filter paper was exposed onto X-ray film for 4 to 5 weeks. The radioactive spots were identified with authentic samples after spraying with Ninhydrin reagent. Figure 1 shows a diagrammatic representation of the chromatogram.

For separation of L-5-HTP metabolites, thin layer chromatography was employed. Radioactive materials were spotted onto cellulose plates (E. Merck, F254, 0.1 mm thickness), and developed with a solvent system composed of n-butanol : acetic acid : distilled water (4 : 1 : 1) at room temperature. Detection of radioactive spots on TLC plates was carried out with both radioscanogram (Packard Model 7201) and autoradiograms were obtained after exposing the plates onto the X-ray film for 4 weeks. Co-chromatography with authentic compounds and color reaction with the Ehrlich reagent served for identification of each radioactive spot.

Determination of radioactivity: The identified radioactive spots on the chromatograms were transferred into counting vials. After shaking with 0.5 ml of $10^{-8} \text{ N HCl}$ for 10 min, 10 ml of the liquid scintillator composed of 8 g of PPO, 200 mg of dimethyl-POPOP, 200 ml of toluene and 800 ml of dioxane was added. The radioactivity was measured using a Packard Model 3380 Liquid Scintillation Spectrometer.

RESULTS

Figures 2 to 5 show representative autoradiograms of L-DOPA-2-14C from various
regions of the brain of cats pretreated with MK-486.

One hr after dosing of L-DOPA-$^{14}$C, there was a considerable uptake of radioactivity in the cat brain and most of this radioactivity was localized in the gray matter, with only a trace amount in the white matter. In the gray matter, the highest uptake was observed in the caudate nucleus, putamen, pallidum, raphe nucleus and substantia nigra. A high uptake of radioactivity was evident in the hypothalamus, followed by the thalamus, cerebellum and cerebral cortex.

At 6 hr after administration, the radioactivity was highly retained in the caudate nucleus, putamen and pallidum, while the radioactivity in the other regions was undetectable.

Autoradiograms obtained from cats after administration of L-5-HTP-3-$^{14}$C (i.v.) are shown in Figures 6 to 8.

Thirty min after administration, the evidence of a high radioactivity indicated an efficient transportation of the amino acid into the brain, particularly in the gray matter. The highest uptake was observed in the hypothalamus and caudate nucleus, followed by the putamen and pallidum. It was observed that, at 3 hr after injection, a high radioactivity was maintained in the hypothalamus, caudate nucleus, raphe nucleus, substantia nigra, pallidum, inferior olivaris, and putamen. The radioactivity in the raphe nucleus was markedly increased.

![Autoradiograms showing the distribution of radioactivity in the brain of the cat pretreated with MK-486 after oral administration of L-DOPA-$^{14}$C](image-url)
compared with 30 min after injection, while concentrations in the cerebral cortex, cerebellum and thalamus were markedly decreased. No further radioactivity was detected in the white matter.

Since radioactivities of L-DOPA and L-5-HTP were clearly localized at 1 and 3 hr after administration, respectively, quantitative determinations of the main metabolites of both amino acids were carried out at corresponding periods. When L-DOPA$^{14}$C was given to the MK-486 treated cat, an appreciable amount of radioactivity was observed in the pons and midbrain (Table 1). A high concentration of dopamine was detected in the caudate nucleus (3.08 \(\mu\)g/g) and hypothalamus (1.74 \(\mu\)g/g). In the other regions, only small quantities of dopamine (less than 0.1 \(\mu\)g/g) were formed. Considerable amounts of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), which were formed via oxidation of dopamine, were found in the hypothalamus, and pons and midbrain. Only small amounts of noradrenaline (less than 0.1 \(\mu\)g/g) were detected in every region of the cat brain. A high concentration of DOPA and considerable amounts of 3-O-methyl DOPA were found in the regions tested.

Radioactive materials derived from L-5-HTP$^{14}$C in the brain were analyzed at 3 hr.

![Autoradiograms showing the distribution of radioactivity in the brain of the cat pretreated with MK-486 after oral administration of L-DOPA$^{14}$C](image)

**Fig. 3.** Autoradiograms showing the distribution of radioactivity in the brain of the cat pretreated with MK-486 after oral administration of L-DOPA$^{14}$C.
after injection. The concentration of radioactivity in the caudate nucleus and hypothalamus was considerably higher than in the thalamus, cerebellum and medulla oblongata. L-5-HTP, serotonin and 5-hydroxyindole-3-acetic acid (5-HIAA) were identified and estimated,

![Fig. 4. Autoradiograms showing the distribution of radioactivity in the brain of the cat pretreated with MK-486 after oral administration of L-DOPA-\textsuperscript{14}C](image)

![Fig. 5. Autoradiograms showing the distribution of radioactivity in the brain of the cat pretreated with MK-486 after oral administration of L-DOPA-\textsuperscript{14}C](image)
FIG. 6. Autoradiograms showing the distribution of radioactivity in the brain of the cat after intravenous administration of L-5-HTP-14C.

FIG. 7. Autoradiograms showing the distribution of radioactivity in the brain of the cat after intravenous administration of L-5-HTP-14C.
Fig. 8. Autoradiograms showing the distribution of radioactivity in the brain of the cat after intravenous administration of L-5-HTP-$^14$C

Table 1. Concentrations of L-DOPA and metabolites in different regions of cat brain pretreated with MK-486 1 hr after oral administration of L-DOPA-$^14$C

| Regions             | Concentration (μg/g tissue) of L-DOPA-$^14$C | 3-O-methyl DOPA-$^14$C | DA-$^2^1$ | NA-$^2^1$ | DOPAC-$^2^1$ | HVA-$^2^1$
|---------------------|---------------------------------------------|------------------------|-----------|-----------|-------------|----------
| Cerebral cortex     | 4.68                                        | 1.43                   | 2.72      | 0.04      | 0.01        | 0.16     | 0.10     
| Cerebellum          | 5.61                                        | 2.18                   | 2.23      | 0.09      | 0.03        | 0.26     | 0.53     
| Caudate nucleus     | 9.95                                        | 2.26                   | 2.44      | 3.08      | 0.09        | 0.64     | 0.75     
| Amygdala            | 7.50                                        | 3.20                   | 2.29      | 0.62      | 0.08        | 0.42     | 0.42     
| Hypothalamus        | 8.73                                        | 1.87                   | 2.15      | 1.74      | 0.16        | 1.12     | 1.44     
| Thalamus            | 7.34                                        | 2.39                   | 1.93      | 0.35      | 0.04        | 0.32     | 0.78     
| Hippocampus         | 4.28                                        | 2.23                   | 1.37      | 0.18      | n.d.        | 0.16     | 0.27     
| Pons and midbrain   | 12.32                                       | 3.72                   | 2.07      | 0.39      | 0.15        | 0.63     | 1.66     
| Medulla oblongata   | 4.39                                        | 1.62                   | 1.45      | 0.25      | 0.08        | 0.26     | 0.65     

1) calculated as μg equivalent to L-DOPA/g tissue
2) calculated as μg/g tissue from radioactivity value in one cat
as shown in Table 2. A higher concentration of L-5-HTP was found in the cerebral cortex and hippocampus, while only small amounts were observed in the hypothalamus. On the contrary, the larger amounts of the radioactive material (up to 60%) in the hypothalamus and the medulla oblongata were identified as serotonin, i.e. 1.09 μg/g in the former and 0.63 μg/g in the latter. 5-HIAA, which is an end product of L-5-HTP metabolism, was rich in the hypothalamus (0.98 μg/g), caudate nucleus (0.74 μg/g) and pons and midbrain (0.70 μg/g), whereas the concentration of this metabolite in the other regions was low.

**DISCUSSION**

Autoradiograms of the cat brain showed that radioactivities of 14C-labeled L-DOPA and L-5-HTP were efficiently transferred into various regions of the brain. Among these, preferential uptake of both radioactivity was observed in the caudate nucleus and hypothalamus. At 6 hr after L-DOPA administration, the radioactivity remained in the caudate nucleus, putamen and pallidum, despite disappearance from the other regions. In the case of L-5-HTP-14C, radioactivity accumulated in the hypothalamus, raphe nucleus, substantia nigra, caudate nucleus and putamen at 3 hr after administration. These findings are in agreement with observations on the physiologic localization of dopamine and serotonin, as described respectively by Bertler and Rosengren (14) and Kuntzman et al. (15).

Tsukada et al. (16) carried out similar studies using monkeys and their findings were much the same as ours reported herein. In their report, however, it was demonstrated that both radioactivities were found in the inferior olivaris of the monkey, whereas we observed no radioactivity of 14C-L-DOPA in this region of the cat. Further investigation is required to clarify this discrepancy in relation to possible species differences of distribution of transmitters.

It was also shown that the most prominent region containing dopamine was the caudate nucleus, and serotonin levels were high in the hypothalamus and low in the caudate nucleus. Since these regions possess relatively extensive activities of L-aromatic amino acid decarboxy-
lase (15), it has been assumed that L-DOPA and L-5-HTP are readily convertible to the corresponding amines, in these regions of the brain stem.

In the present study, MK-486 was used to enhance the brain uptake of L-DOPA, through the inhibition of peripheral L-aromatic amino acid decarboxylase (7, 12, 17). The fact that the dopamine-level was markedly enhanced in the brain, particularly in the caudate nucleus, suggests that this inhibitor did not affect the enzyme in the CNS.

Significant amounts of 3-0-methyl DOPA were found in all parts of the brain examined. Since localization of catechol-O-methyl transferase (COMT) in the CNS is limited to the caudate nucleus and hypothalamus (18), the ubiquitous existence of this amino acid in the brain tissue cannot be attributed to the intracerebral formation from L-DOPA. Unpublished results in this laboratory showed that 3-O-methyl DOPA easily passes the blood brain barrier. Accordingly, greater amounts of this amino acid might be transferred into the brain tissues from the periphery after methylation of L-DOPA by extracerebral COMT.

As a conclusion, our present results indicate that, even after being administered exogenously, L-DOPA and L-5-HTP were efficiently converted to the corresponding amines, dopamine and serotonin, in the regions inherently rich in amine contents.

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