Brain Extraction of 4-(4-Chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]pyridinium Ion (HPP⁺), a Neurotoxic Metabolite of Haloperidol: Studies Using [³H]HPP⁺

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ABSTRACT—Tritium-labeled 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]pyridinium ion (HPP⁺) was synthesized enzymatically from [³H]haloperidol using rat liver microsomal preparations, and using prepared [³H]HPP⁺, the passage of HPP⁺ into the brain was investigated. Consequently, HPP⁺ showed a moderate brain uptake index, indicating that it is able to permeate the blood-brain barrier. Furthermore, HPP⁺ was detected in murine brains after being intravenously injected. These results suggested that HPP⁺, produced mainly in the liver, is taken up into the brain and induces damage to brain dopaminergic neurons.

Keywords: HPP⁺, Blood-brain barrier, Dopaminergic neuronal toxicity

Since 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]pyridinium ion (HPP⁺), a cationic metabolite of haloperidol, was detected in the brains of patients who had received haloperidol chronically (1), and HPP⁺ has shown cytotoxicity toward dopaminergic neurons in vitro (2), it is suggested that HPP⁺ participates in tardive motor dysfunction induced by long-term treatment with haloperidol. Furthermore, in vitro studies have also shown that HPP⁺ is mainly produced by the microsomal-catalyzed oxidation of haloperidol with cytochrome P450 (CYP) which is distributed principally in the liver (3, 4). Therefore, it has been suggested that HPP⁺ is produced in the liver and taken up into the brain. However, thus far the permeability of HPP⁺ through the blood-brain barrier (BBB) has not been investigated.

In this study, to examine the permeability of HPP⁺ through the BBB, [³H]HPP⁺ was synthesized and the brain uptake index (BUI) of HPP⁺ was determined in rats. Furthermore, HPP⁺ detection was performed in the brain after intravenous injection of HPP⁺ into mice.

Hepatic microsomal fractions were prepared by the conventional centrifugation technique (5), and then [³H]HPP⁺ was prepared from [³H]haloperidol by a co-factor generating system for non-radioactive HPP⁺ (6), with some minor modifications. Briefly, [³H]haloperidol (14.0 Ci/mmol; New England Nuclear Life Sciences Products, Inc., Boston, MA, USA) and the microsomal fraction were added to phosphate buffer containing G6P-nicotinamide adenine dinucleotide phosphate monosodium salt (2 G6d mol), D-glucose-6-phosphate monosodium salt (10 G6d mol), glucose-6-phosphate dehydrogenase (10 units) and magnesium chloride (20 G6d mol). After 2-h incubation, acetonitrile was added to stop the reaction, the reaction mixture was centrifuged and the supernatant concentrated by removing volatile substances under reduced pressure. The residual solution was applied to HPLC (SHIMADZU LC-6A pump, attached to an SPD-6A UV spectrophotometric detector (248 nm; Shimadzu Co., Kyoto), YMC-Pack Pro C18 column (4.6 x 150 mm; YMC Co., Ltd., Kyoto), 37:63 (v/v) mixture of acetonitrile and 10 mM phosphate buffer (pH 3.0, containing 5 mM sodium 1-octanesulfonate), with flow rates of 1.0 ml/min: retention time / 3d 18.0 min for HPP⁺ and 12.5 min for haloperidol); and the fraction corresponding to [³H]HPP⁺ was collected (radiochemical yield: 4.1%). The radiochemical purity of [³H]HPP⁺ produced was more than 98% as determined by TLC (chloroform : methanol = 5:1, Rf = 0.30).

To evaluate the BBB permeability, the BUI of [³H]HPP⁺ was measured using the double isotope-single injection

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method (7). BUI is the percent ratio of the first-pass extraction of the test radioligand relative to that of \[^{14}C\]butanol, which is assumed to be freely diffusible. Briefly, anesthetized male Wistar rats (200 – 210 g) were injected with 200 \(\mu\)l of saline containing a mixture of \[^{14}C\]butanol (14.8 kBq) and \[^{1}H\]HPP\(^*\) (11.1 kBq) as a single bolus via the right common carotid artery. This injection was rapid and was completed within 0.5 s. The rats were killed by decapitation 15 s later, and the temporoparietal cerebral cortex ipsilateral to the carotid injection was immediately removed. The tissues were treated with NCS-II tissue solubilizer (Amersham, Little Chalfont, UK), and then acetic acid was added to neutralize the solution. The samples were mixed with 5 ml of liquid scintillation fluid (Clea-sol I), and the radioactivity was measured using simultaneous mixed with 5 ml of liquid scintillation fluid (Clea-sol I), and the radioactivity was measured using simultaneous mixed with 5 ml of liquid scintillation fluid (Clea-sol I), and the radioactivity was measured using simultaneous mixed with 5 ml of liquid scintillation fluid (Clea-sol I), and the radioactivity was measured using simultaneous mixed with 5 ml of liquid scintillation fluid (Clea-sol I), and the radioactivity was measured using simultaneous mixed with 5 ml of liquid scintillation fluid (Clea-sol I), and the radioactivity was measured using simultaneous mixed with 5 ml of liquid scintillation fluid (Clea-sol I), and the radioactivity was measured using simultaneous mixed with 5 ml of liquid scintillation fluid (Clea-sol I), and the radioactivity was measured using simultaneous mixed with 5 ml of liquid scintillation fluid (Clea-sol I), and the radioactivity was measured using simultaneous mixed with 5 ml of liquid scintillation fluid (Clea-sol I), and the radioactivity was measured using simultaneous mixed with 5 ml of liquid scintillation fluid (Clea-sol I), and the radioactivity was measured using simultaneous mixed with 5 ml of liquid scintillation fluid (Clea-sol I), and the radioactivity was measured using simultaneous.

**Table 1.** Brain uptake index (BUI) of HPP\(^*\) and Zn-EDTA

| Compound   | BUI (% of \[^{14}C\]butanol) |
|------------|-----------------------------|
| HPP\(^*\)  | 9.0 ± 2.1 (n = 6)            |
| Zn-EDTA    | 0.6 ± 0.3 (n = 5)            |

Each value represents the mean ± S.D.

HPP\(^*\) showed relatively higher BUI than \[^{65}Zn\]Zn-EDTA, a non-BBB permeable compound (8) (Table 1).

In addition, the brain uptake of HPP\(^*\) administered peripherally was measured. HPP\(^*\) chloride was synthesized as previously described (9). Briefly, haloperidol (Research Biochemicals International, Natick, MA, USA) and 35% HCl in distilled toluene were refluxed to form the intermediate product 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydropyridine (HPTP). HPTP was subsequently converted into the HCl salt, which was allowed to react with platinum charcoal in distilled methanol at room temperature under dry argon to obtain HPP\(^*\) chloride (mp 208 – 210\(^\circ\)C). The \(^1\)H nuclear magnetic resonance spectrum was identical to that reported (10). Eor C\(_{21}\)H\(_{38}\)ClF\(_7\)N'O·Cl\(_2\), calculated: C: 64.63 H: 4.65 N: 3.59; found C: 62.59 H: 4.76 N: 3.44. MS m/z 354 (calculated for 354). Male ddY mice (30 g) were injected intravenously with 0.5 \(\mu\)g (1.3 nmol) of HPP\(^*\) chloride in saline solution. At specified time points, the mice were decapitated and their brains rapidly removed. The brains were washed three times with 5 ml of saline each time. HPP\(^*\) was extracted, according to the method of Igarashi and Castagnoli. (11). Briefly, the brain was homogenized in 2 volumes of 1.15% KCl solution with a Polytron homogenizer. The homogenate was mixed with an equal volume of methanol containing 2% acetic acid, and this mixture was centrifuged at 10,000 \(\times\)g for 10 min. Quantitative analysis of HPP\(^*\) in the obtained supernatant was performed with the HPLC isolation method (SHIMADZU LC-10AD pump, attached to a PF-10A XL fluorescence detector (Ex 302 nm, Em 372 nm; Shimadzu), YMC-Pack Pro C18 column (4.6 × 150 mm), 40:60 (\(v/v\)) mixture of acetonitrile and 10 mM phosphate buffer (pH 3.0, containing 5 mM sodium 1-octanesulfonate), with flow rates of 1.0 ml/min: retention time – 8.5 min).

The HPP\(^*\) uptake into the brain after an intravenous injection of HPP\(^*\) into mice is summarized in Fig. 1. HPP\(^*\) entered the brain rapidly, and a high uptake (57.8 ± 13.2 pmol/g brain) was observed at the initial sampling time of 1 min. Furthermore, when the brain uptake ratio to the injected dose was calculated at 5 min post-injection, HPP\(^*\) showed high permeability to the brain compared to radioiodinated serum albumin (\[^{131}I\]RISA) as negative reference (12) (HPP\(^*\): 3.4 ± 0.8% injected dose/g. \[^{131}I\]RISA: 0.64 ± 0.05% injected dose/g).

A compound is transported passively across the BBB from the blood to the brain if it does not penetrate the BBB by a specific system, such as carrier-mediated penetration. The BBB permeability of a passively transported compound correlates highly with its lipophilicity. Thus, the lipophilicity of HPP\(^*\) was assessed by 1-octanol-phosphate buffer (pH 7.4) extraction as described previously (13).

Briefly, \[^{1}H\]HPP\(^*\) was mixed with 2.0 ml each of 1-
octanol and 0.1 M phosphate buffer (pH 7.4) in test tubes. The aqueous phase were pre-saturated with octanol by equilibration overnight at 25°C before the experiments. The tubes were vortexed and incubated for 1 h at 25°C, and then centrifuged. Aliquots of 800 µl of each phase were removed and the radioactivity measured using a liquid scintillation counter. The partition coefficient (P value) was determined by calculating the ratio of radioactivity in 1-octanol to that in phosphate buffer solution.

HPP⁺ showed a log P value of 0.81 ± 0.04, which is approximately the same order as previously calculated using the spectrophotometry method (14). This result supports the possibility that HPP⁺ diffuses passively into the brain to some extent since it has been reported that compounds ranging in log P from 0.9 to 2.5 can pass freely through the BBB by passive diffusion (15).

In conclusion, these observations demonstrated that HPP⁺, a neurotoxic metabolite of haloperidol, could permeate from the blood into the brain because of its lipophilicity. Thus, it is suggested that tardive dyskinesia manifesting during chronic haloperidol treatment is, in part, attributable to HPP⁺ produced in the liver and taken up into the brain.

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