Evaluation of $^3$H-Paroxetine as a Radioligand for in vivo Study of 5-Hydroxytryptamine Uptake Sites in Mouse Brain

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The distribution of radioactivity in the mouse brain after intravenous administration of $^3$H-paroxetine was in the order (highest to lowest) hypothalamus $>$ cerebral cortex $>$ cerebellum. The radioactivity in the hypothalamus and cerebral cortex after injection of $^3$H-paroxetine was significantly decreased by treatment with 6-nitroquipazine or paroxetine. HPLC and TLC analyses show that no radioactive metabolites were found in the mouse brain 3 h after intravenous administration of $^3$H-paroxetine. The present results indicate that $^3$H-paroxetine would be a suitable radioligand for in vivo study of 5-HT uptake sites in mouse brain.

Key Words: tritium-paroxetine, 5-hydroxytryptamine uptake sites, in vivo binding, mouse brain

1. Introduction

High affinity binding sites for the tricyclic antidepressant $^3$H-imipramine have been described in the brain and platelets of several mammalian species. These binding sites have been related to the uptake sites for 5-hydroxytryptamine (5-HT; serotonin). It has been reported that the densities of $^3$H-imipramine binding sites are significantly decreased in the platelets of depressed patients, in the frontal cortex of suicide victims and in the brain of Alzheimer's disease/senile dementia of Alzheimer type.

The in vivo labelling of 5-HT$_2$ receptors in the intact human (or baboon) brain using positron emission tomography (PET) has recently been demonstrated, but has not been reported for 5-HT uptake sites in the intact human brain. However, the in vivo labelling of 5-HT uptake sites in the rat (or mouse) brain after administration of $^3$H-cyanoimipramine, a potent 5-HT uptake inhibitor, has been reported. The in vivo study of 5-HT uptake sites in the intact human brain using PET is, therefore, of great interest due to their possible biological role in the affective disorders such as depression.

Paroxetine ((-) trans-4-(p-fluorophenyl)-3-[3, 4-methylenedioxyphenoxy-methyl] piperidine) is a very potent and selective inhibitor of 5-HT uptake. $^3$H-Paroxetine has been used in the binding assays in vitro, and it is described that the binding sites labelled by $^3$H-paroxetine is associated with the neuronal 5-HT transporter complex. $^3$H-Paroxetine might be a suitable radioligand for in vivo study of 5-HT uptake sites in brain, since paroxetine is a more potent 5-HT uptake inhibitor than cyanoimipramine. In the present study, we evaluated the potential of $^3$H-paroxetine as a radiotracer for in vivo labelling of 5-HT uptake sites in mouse brain. The results of this study were presented at the Proceedings of Third Annual Meeting of the Japanese Society for the Study of Xenobiotics, Osaka, 1988.

2. Materials and Methods

2.1 Animals

Male ddy mice weighing 30–35 g were used. The animals were housed in a group of 10 animals in a cage. They were maintained under standard conditions (light on from 6:00 to 22:00)
to 18:00 h, room temperature 23±1 °C, humidity 55±5%) with free access to food and water.

2.2 Materials

$^3$H-Paroxetine (854.7 and 1032.3 GBq/mmol) was purchased from New England Nuclear (Boston, MA, U.S.A.). Paroxetine HCl was donated by Beecham Pharmaceutical Ltd. (Surrey, U.K.). Imipramine HCl (Wako Pure Chemical Ltd., Tokyo, Japan), desipramine HCl (Sigma Chemical Co., St Louis, MO, U.S.A.) and GBR 12909 (Research Biochemicals Inc., Natick, MA, U.S.A.) were used. 6-Nitro-2-(1-piperazinyl) quinoline maleate (6-nitroquipazine maleate) was synthesized in our laboratory. Other chemicals were purchased commercially.

2.3 Distribution of radioactivity in mouse brain after intravenous administration of $^3$H-paroxetine

Mice were intravenously injected with 0.2 ml of $^3$H-paroxetine solution (about 100 kBq). The mice were killed by decapitation 0.5, 1, 3 and 5 h after injection of the radiotracer. Blood, hypothalamus, cerebral cortex and cerebellum were quickly removed and weighed, each sample being incinerated by a sample oxidizer (Aloka, ASC-113), and the percentage of injected dose per gram tissue (% dose/g) in each sample was determined by a liquid scintillation counter (Aloka, LSC-1000). In order to determine the degree of specific binding, 6-nitroquipazine, a potent 5-HT uptake inhibitor was intraperitoneally injected in the mice 30 min before intravenous administration of $^3$H-paroxetine (about 100 kBq). The distribution of radioactivity in each tissue 0.5, 1, 3 and 5 h after injection of the radiotracer was determined as described above. 6-Nitroquipazine solution (10 mg/kg, expressed as the free base) was prepared by saline in a volume of 0.1 ml/10 g of body weight.

2.4 Dose effects of paroxetine and 6-nitroquipazine on the distribution of $^3$H-paroxetine in mice

Mice were intravenously injected with 0.2 ml of $^3$H-paroxetine solution (about 100 kBq) containing various doses of paroxetine (0, 0.01, 0.1 and 1 mg/kg) or 6-nitroquipazine (0, 0.01, 0.1, 1 and 10 mg/kg). The mice were killed by decapitation 3 h after administration of $^3$H-paroxetine. The distribution of radioactivity in the blood, hypothalamus, cerebral cortex and cerebellum was determined as described above.

2.5 Effects of monoamine uptake inhibitors on the distribution of $^3$H-paroxetine in mice

Mice were intravenously injected with 0.2 ml of $^3$H-paroxetine (about 100 kBq) 30 min after intraperitoneal injection of imipramine (1 mg/kg), desipramine (1 mg/kg) or GBR 12909 (1 mg/kg). The distribution of radioactivity in the blood, hypothalamus, cerebral cortex and cerebellum 3 h after injection of the radiotracer was determined as described above.

2.6 Stability of $^3$H-paroxetine in the mouse brain

About 200 kBq of $^3$H-paroxetine solution was intravenously injected into the mouse. Three hours after injection of the radiotracer, the mouse was killed by decapitation. The brain was quickly removed, and the brain was homogenized with 1 ml of phosphate buffer (0.2 M, pH 7.4), and 0.5 ml of ethanol containing 1 mg paroxetine was added to 0.5 ml of the brain homogenate, then radioactive materials were extracted. The extractable materials were analyzed by high performance liquid chromatography (HPLC) (Column; Shim-pack CLC-ODS (5 μm, 6.0 mm I.D. x 15 cm), Mobile phase; acetonitrile: 1% aqueous triethylamine acetate (pH 4.0) = 4:6, Flow rate; 2 ml/min, Detector; UV (254 nm) and thin layer chromatography (TLC) (silicagel; chloroform:triethylamine = 20:1, Rs value = 0.14) as described previously.

2.7 Statistics

The statistical evaluation of multigroup data was performed by a one-way analysis of variance (ANOVA) followed by the Scheffe's
3. Results

Figure 1A shows the time course of radioactivity in the blood, hypothalamus, cerebral cortex and cerebellum after intravenous administration of $^3$H-paroxetine. These brain regions were selected for study as it has been shown to have high, intermediate and low levels of $^3$H-paroxetine binding, respectively. The distribution of radioactivity in the three brain regions was almost similar 0.5 h after injection of the radiotracer. Radioactivity in the hypothalamus was decreased slowly whereas that in the cerebral cortex was decreased moderately. On the other hand, the radioactivity in the blood and cerebellum was reduced rapidly. Furthermore, the radioactivity in the blood, hypothalamus and cerebral cortex after intravenous administration of $^3$H-paroxetine was significantly decreased by pretreatment with 6-nitroquipazine (10 mg/kg), a potent selective 5-HT uptake inhibitor, whereas that in the cerebellum was not decreased, as shown in Fig. 1B. The time course of radioactivity in the hypothalamus, cerebral cortex and cerebellum was almost the same with the 6-nitroquipazine pretreated mice.

The time course of the ratios of hypothalamus to cerebellum and cerebral cortex to cerebellum binding is shown in Fig. 2. These ratios could reflect those of the total binding to the non-specific binding and free ligand, since the cerebellum has very low levels of $^3$H-paroxetine binding. As shown in Fig. 2, these ratios were significantly reduced by pretreatment with 6-nitroquipazine.

![Graph showing time courses of radioactivity in the A) control and B) 6-nitroquipazine (10 mg/kg, 30 min before)-pretreated mice after intravenous administration of $^3$H-paroxetine. Radioactivity in the blood (O), hypothalamus (●), cerebral cortex (■) and cerebellum (□) was expressed as percentage of injected dose per gram tissue (% dose/g tissue). Values are presented as an average±S.D. of three mice in each point.](image)
Radioactivity in the hypothalamus and cerebral cortex 3 h after intravenous administration of $^3$H-paroxetine was significantly decreased by coinjection of paroxetine or 6-nitroquipazine, in a dose dependent manner (Fig. 3 and Fig. 4). Furthermore, the hypothalamus/cerebellum and cerebral cortex/cerebellum ratios were also decreased by the treatment with paroxetine or 6-nitroquipazine, in a dose dependent manner.

Radioactivity in the hypothalamus and cerebral cortex 3 h after intravenous administration of $^3$H-paroxetine was slightly decreased by pretreatment with imipramine (1 mg/kg), but not by pretreatment with desipramine (1 mg/kg) or GBR 12909 (1 mg/kg), as shown in Table 1.

HPLC and TLC analyses of the brain extracts showed that brain radioactivity following $^3$H-paroxetine administration was found to be due to unmetabolized $^3$H-paroxetine (Fig. 5 and Fig. 6).

4. Discussion

The present results indicate that $^3$H-paroxetine would be a suitable radiotracer for in vivo study of 5-HT uptake sites in mouse brain. This conclusion is based on the follow-
Fig. 4 (A) Distribution of radioactivity in the mice 3 h after intravenous administration of \(^3\)H-paroxetine with various doses of 6-nitroquipazine. Radioactivity in the blood (□), hypothalamus (●), cerebral cortex (■) and cerebellum (○) was expressed as % dose/g tissue.

(B) The hypothalamus/cerebellum (●) and cerebral cortex/cerebellum (■) ratios are from the data (A).

Values are presented as an average ± S.D. of three mice in each point. *P<0.05, **P<0.01 when compared with control.

Table 1 Effects of monoamine uptake inhibitors on the distribution of \(^3\)H-paroxetine in the mouse brain

<table>
<thead>
<tr>
<th>% Dose/g</th>
<th>Blood</th>
<th>Hypothalamus</th>
<th>Cerebral cortex</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.816±0.23</td>
<td>3.25±0.28</td>
<td>2.07±0.28</td>
<td>0.967±0.20</td>
</tr>
<tr>
<td>Imipramine (1 mg/kg)</td>
<td>0.613±0.02</td>
<td>2.86±0.34</td>
<td>1.88±0.16</td>
<td>0.834±0.03</td>
</tr>
<tr>
<td>Desipramine (1 mg/kg)</td>
<td>0.663±0.06</td>
<td>3.12±0.20</td>
<td>2.04±0.09</td>
<td>1.03±0.09</td>
</tr>
<tr>
<td>GBR 12909 (1 mg/kg)</td>
<td>0.703±0.06</td>
<td>3.45±0.38</td>
<td>2.13±0.31</td>
<td>1.02±0.04</td>
</tr>
</tbody>
</table>

Three mice in each group; average ± S.D.

Fig. 5 HPLC analysis of radioactive materials in the mouse brain 3 h after intravenous administration of \(^3\)H-paroxetine.

Column; Shim-pack CLC-ODS, Mobile phase: acetonitrile: 1% aqueous triethylamine acetate (pH 4.0), Flow rate; 2 ml/min, Detector; UV (254 nm), Retention time of paroxetine (4.6 min).

Fig. 6 TLC analysis of radioactive materials in the mouse brain 3 h after intravenous administration of \(^3\)H-paroxetine.

●: Detected by UV lump, Solvent system: chloroform: triethylamine=20:1, \(R_f\) value = 0.14
ing findings: (1) High accumulation of radioactivity in the mouse brain was shown after intravenous administration of \(^3H\)-paroxetine. The radioactivity in the brain after injection of \(^3H\)-paroxetine was in the order (highest to lowest) hypothalamus > cerebral cortex > cerebellum. This is almost the same order for \(^3H\)-paroxetine binding in vitro\(^{27}\). Since the cerebellum has a very low density of \(^3H\)-paroxetine binding\(^{27}\), the radioactivity in the cerebellum can, therefore, be used as non-specific binding and free ligand. Also, the in vivo binding of \(^3H\)-paroxetine in the cerebellum was not altered by pretreatment with 6-nitroquipazine (10 mg/kg) (Fig. 1), indicating that the radioactivity in the cerebellum was due to the non-specific binding and free ligand. Furthermore, the hypothalamus/cerebellum ratio was more higher than the cerebral cortex/cerebellum ratio. (2) The distribution of radioactivity in the hypothalamus and cerebral cortex after injection of \(^3H\)-paroxetine was significantly decreased by pretreatment with 6-nitroquipazine and decreased by coadministration of paroxetine or 6-nitroquipazine, in a dose dependent manner. Furthermore, the radioactivity in the hypothalamus and cerebral cortex 3 h after administration of \(^3H\)-paroxetine was slightly reduced by pretreatment with imipramine (an inhibitor of both 5-HT and NE uptake), whereas not by pretreatment with desipramine (a NE uptake inhibitor)\(^{39}\) or GBR 12909 (a DA uptake inhibitor)\(^{39}\). It was found that imipramine had a very weak property for inhibition of \(^3H\)-paroxetine binding in vivo as compared with paroxetine and 6-nitroquipazine. The dose of desipramine and GBR 12909 used in the present study seems to be sufficient for inhibiting NE or DA uptake respectively\(^{191,281,291}\). It was found that \(^3H\)-paroxetine binding was more selective for 5-HT uptake inhibitors than NE or DA uptake inhibitors. This is also supported by the in vitro data\(^{193}\) of Hyttel. (3) From HPLC and TLC analyses of radioactivity in the brain extracts, it was found that \(^3H\)-paroxetine was stable in the mouse brain 3 h after intravenous administration.

More recently, Scheffel and Hartig have presented that \(^3H\)-paroxetine bound specifically to 5-HT uptake sites in vivo\(^{33}\). Taken together, the present results indicate that \(^3H\)-paroxetine would be a suitable radioligand for in vivo labelling of 5-HT uptake sites in mouse brain. Furthermore, it would be possible to study in vivo the 5-HT uptake sites in the intact human brain using \(^18F\)-paroxetine and PET, if high specific activity \(^18F\)-paroxetine could be prepared.

References

12) Wong, D.F., Lever, J.R., Hartig, P.R.,

要　旨

マウス脳内セロトニン再吸収部位の in vivo 研究用ラジオリガンドとしての 3H-Paroxetine の評価

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3H-paroxetine 投与後の脳各部位における放射能分布は、視床下部において最も高く、つぎに大脳皮質において中程度であり、小脳は最も少なかった。また、視床下部および大脳皮質における放射能分布は 6-nitroquipazine と paroxetine の投与によって有意に減少した。以上の結果より、3H-paroxetine はマウス脳内セロトニン再吸収部位のインビボ研究に有用なラジオリガンドになることが分かった。

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