Evaluation of ^3H -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 ^3H -paroxetine was in the order (highest to lowest) hypothalamus > cerebral cortex > cerebellum. The radioactivity in the hypothalamus and cerebral cortex after injection of ^3H -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 ^3H -paroxetine. The present results indicate that ^3H -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 ^3H -imipramine have been described in the brain and platelets of several mammalian species⁽¹⁻⁴⁾. These binding sites have been related to the uptake sites for 5-hydroxytryptamine (5-HT; serotonin)^(5,6). It has been reported that the densities of ^3H -imipramine binding sites are significantly decreased in the platelets of depressed patients^(7,8), in the frontal cortex of suicide victims⁽⁹⁾ and in the brain of Alzheimer's disease/senile dementia of Alzheimer type⁽¹⁰⁾.

The *in vivo* labelling of 5-HT₂ receptors in the intact human (or baboon) brain using positron emission tomography (PET) has recently been demonstrated^(11,12), 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 ^3H -cyanoimipramine, a potent 5-HT uptake inhibitor, has been reported^(13,14). 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⁽¹⁵⁻¹⁹⁾. ^3H -Paroxetine has been used in the binding assays *in vitro*⁽²⁰⁻²³⁾, and it is described that the binding sites labelled by ^3H -paroxetine is associated with the neuronal 5-HT transporter complex⁽²³⁾. ^3H -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^(17,18). In the present study, we evaluated the potential of ^3H -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⁽²⁴⁾.

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

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to 18:00 h, room temperature $23 \pm 1^\circ\text{C}$, humidity $55 \pm 5\%$) with free access to food and water.

2.2 Materials

^3H -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 ^3H -paroxetine

Mice were intravenously injected with 0.2 ml of ^3H -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 inhibitor²⁵⁾ (10 mg/kg) was intraperitoneally injected in the mice 30 min before intravenous administration of ^3H -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 ^3H -paroxetine in mice

Mice were intravenously injected with 0.2 ml of ^3H -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 ^3H -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 ^3H -paroxetine in mice

Mice were intravenously injected with 0.2 ml of ^3H -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 ^3H -paroxetine in the mouse brain

About 200 kBq of ^3H -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. $\times$ 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, R_f value=0.14) as described previously²⁶⁾.

2.7 Statistics

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

test for multiple comparisons. The criterion for significance was $P < 0.05$.

3. Results

Figure 1 A shows the time course of radioactivity in the blood, hypothalamus, cerebral cortex and cerebellum after intravenous administration of ^3H -paroxetine. These brain regions were selected for study as it has been shown to have high, intermediate and low levels of ^3H -paroxetine binding, respectively²⁷⁾. 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 ^3H -paroxetine was significantly decreased by pretreatment with 6-nitroquipazine (10 mg/kg), a potent selective 5-HT uptake inhibitor²⁵⁾, whereas that in the cerebellum was not decreased, as shown in Fig. 1 B. 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 ^3H -paroxetine binding. As shown in Fig. 2, these ratios were significantly reduced by pretreatment with 6-nitroquipazine.

Fig. 1 Time courses of radioactivity in the (A) control and (B) 6-nitroquipazine (10 mg/kg, 30 min before)-pretreated mice after intravenous administration of ^3H -paroxetine.

Radioactivity in the blood (○), hypothalamus (●), cerebral cortex (■) and cerebellum (□) was expressed as percentage of injected dose per gram tissue (% dose/g tissue). Values are presented as an average $\pm$ S.D. of three mice in each point.

Fig. 2 Time course of the tissue/cerebellum ratios.

The data in the hypothalamus/cerebellum ratio (●) and cerebral cortex/cerebellum (■) are that from Fig. 1(A). The data in the hypothalamus/cerebellum (○) and cerebral cortex/cerebellum (□) are that from Fig. 1(B). Values are presented as an average $\pm$ S.D. of three mice in each group. * $P < 0.05$, ** $P < 0.01$ when compared with control.

Fig. 3 (A) Distribution of radioactivity in the mice 3 h after intravenous administration of ^3H -paroxetine with various doses of paroxetine. 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 $\pm$ S.D. of three mice in each point. * $P < 0.05$, ** $P < 0.01$ when compared with control.

Radioactivity in the hypothalamus and cerebral cortex 3 h after intravenous administration of ^3H -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 ^3H -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 ^3H -paroxetine administration was found to be due to unmetabolized ^3H -paroxetine (Fig. 5 and Fig. 6).

4. Discussion

The present results indicate that ^3H -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 ^3H -paroxetine with various doses of 6-nitroquipazine. Radioactivity in the blood ($\square$), hypothalamus ($\bullet$), cerebral cortex ($\blacksquare$) and cerebellum ($\circ$) was expressed as % dose/g tissue.

(B) The hypothalamus/cerebellum ($\bullet$) and cerebral cortex/cerebellum ($\blacksquare$) ratios are from the data (A).

Values are presented as an average $\pm$ 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 ^3H -paroxetine in the mouse brain

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

Three mice in each group; average $\pm$ S.D.

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

$\bullet$: Detected by UV lamp, 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 binding²⁷⁾, 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)²⁸⁾ or GBR 12909 (a DA uptake inhibitor)²⁹⁾. 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 respectively¹⁸⁾,²⁸⁾,²⁹⁾. 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* data¹⁸⁾ 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*³⁰⁾. 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 ^{18}F -paroxetine and PET, if high specific activity ^{18}F -paroxetine could be prepared.

References

- 1) Briley, M.S., Raisman, R. and Langer, S.Z.: *Eur. J. Pharmacol.*, **58**, 347-348 (1979)
- 2) Raisman, R., Briley, M.S. and Langer, S.Z.: *Nature*, **281**, 148-150 (1980)
- 3) Paul, S.M., Rehavi, M., Skolnick, P. and Goodwin, F.K.: *Life Sci.*, **26**, 953-959 (1980)
- 4) Langer, S.Z., Javoy-Agid, F., Raisman, R., Briley, M. and Agid, Y.: *J. Neurochem.*, **37**, 267-271 (1981)
- 5) Langer, S.Z., Molet, C., Raisman, R., Dubocovich, M.L. and Briley, M.: *Science*, **210**, 1133-1135 (1980)
- 6) Paul, S.M., Rehavi, M., Rice, K.C., Ittah, Y. and Skolnick, P.: *Life Sci.*, **28**, 2753-2760 (1981)
- 7) Briley, M.S., Langer, S.Z., Raisman, R., Sechter, D. and Zarifian, E.: *Science*, **209**, 303-305 (1980)
- 8) Langer, S.Z., Raisman, R., Sechter, D., Gay, C., Loo, H. and Zarifian, E.: "Frontiers in Biochemical and Pharmacological Research in Depression", (Usdin, E., Asberg, M., Bertilsson, L. and Sjoqvist, F. eds.), pp. 113-125, Raven Press, New York (1984)
- 9) Stanley, M., Virgilio, J. and Gershon, S.: *Science*, **216**, 1337-1339 (1982)
- 10) Marcusson, J.O., Alafuzoff, I., Backstrom, I.T., Ericson, E., Gottfries, C.G. and Winblad, B.: *Brain Res.*, **425**, 137-145 (1987)
- 11) Blin, J., Pappata, S., Kiyosawa, M., Crouzel, C. and Baron, J.C.: *Eur. J. Pharmacol.*, **147**, 73-82 (1988)
- 12) Wong, D.F., Lever, J.R., Hartig, P.R.,

- Dannals, R.F., Villemagne, V., Hoffman, B.J., Wilson, A.A., Ravert, H.T., Links, J.M., Scheffel, U. and Wagner, H.N.: *Synapse*, **1**, 393-398 (1987)
- 13) Hashimoto, K., Inoue, O., Suzuki, K., Yamasaki, T. and Kojima, M.: *Nucl. Med. Biol.*, **14**, 587-592 (1987)
- 14) Wolfe, J., Kreider, M.S., Goodman, C. and Brunswick, D.J.: *J. Pharmacol. Exp. Ther.*, **241**, 196-203 (1987)
- 15) Buus Lassen, J.: *Eur. J. Pharmacol.*, **47**, 351-358 (1978)
- 16) Petersen, E.M., Bechgaard, E., Sortwell, R.J. and Wetterberg, L.: *ibid.*, **52**, 115-119 (1978)
- 17) Hyttel, J.: *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, **6**, 277-295 (1982)
- 18) Maitre, L., Baumann, P.A., Jaekel, J. and Waldmeier, P.C.: "Serotonin in Biological Psychiatry", (Ho, B.T., Schoolar, J.C. and Usdin, E. eds.), pp. 229-246, Raven Press, New York (1982)
- 19) Thomas, D.R., Nelson, D.R., and Johnson, A.M.: *Psychopharmacology*, **93**, 193-200 (1987)
- 20) Mellerup, E.T., Plenge, P. and Engelstoft, M.: *Eur. J. Pharmacol.*, **96**, 303-309 (1983)
- 21) Plenge, P. and Mellerup, E.T.: *Biophys. Acta*, **770**, 22-28 (1984)
- 22) Mellerup, E.T. and Plenge, P.: *Psychopharmacology*, **89**, 436-439 (1986)
- 23) Habert, E., Graham, D., Tahraoui, L., Claustre, Y. and Langer, S.Z.: *Eur. J. Pharmacol.*, **118**, 107-114 (1985)
- 24) Hashimoto, K. and Goromaru, T.: *Xenobio. Metabol. Dispos.*, **3**, 574-575 (1988)
- 25) Vaatstra, W.J., Deiman-Van Aalst, W. M.A. and Eigeman, L.: *Eur. J. Pharmacol.*, **70**, 195-202 (1981)
- 26) Hashimoto, K. and Goromaru, T.: *Kaku Igaku*, **26**, 879-885 (1989)
- 27) De Souza, E.B. and Kuyatt, B.L.: *Synapse*, **1**, 488-496 (1987)
- 28) Lee, C.M., Javitch, J.A. and Snyder, S.H.: *J. Neurosci.*, **2**, 1515-1525 (1982)
- 29) Van der Zee, P., Koger, H.S., Gootjes, J. and Hespe, W.: *Eur. J. Med. Chem.*, **15**, 363-370 (1980)
- 30) Scheffel, U. and Hartig, P.R.: *J. Neurochem.*, **52**, 1605-1612 (1989)

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。