Biochemical Pharmacology, Vol. 38, No. 19, pp. 3169–3173, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

DETERMINATION OF BRAIN CONCENTRATIONS OF 8-HYDROXY-2-(DI-*n*-PROPYLAMINO)TETRALIN BY LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

KENNETH W. PERRY and RAY W. FULLER*
Lilly Research Laboratories, Eli Lilly & Co., Lilly Corporate Center, Indianapolis, IN 46285, U.S.A.

(*Received* 14 *September* 1988; *accepted* 8 *February* 1989)

Abstract—A liquid chromatographic method using electrochemical detection is described for the assay of brain concentrations of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), a centrally acting serotonin agonist selective for the 5HT-1A subtype of serotonin receptors. The method is sensitive to approximately 5 ng/g concentrations. After a 1 mg/kg s.c. dose of 8-OH-DPAT in rats, its concentration in whole brain declined rapidly during the first 4 hr with a half-life of 26 min. At 30 min after a 1 mg/kg s.c. dose of 8-OH-DPAT, concentrations were approximately equal in hypothalamus, striatum, hippocampus, cerebellum and brain stem but were slightly lower in midbrain. 8-OH-DPAT disappeared from hypothalamus, midbrain and hippocampus at similar rates during the first 90 min after a 1 mg/kg s.c. dose. Concentrations of 8-OH-DPAT in whole brain were markedly higher after s.c. than after i.p. administration of 8-OH-DPAT, consistent with earlier data showing 8-OH-DPAT to be more potent when given s.c. than when given i.p. in decreasing brain concentrations of 5-hydroxyindoleacetic acid. Pretreatment with proadifen (SKF-525A), an inhibitor of microsomal drug metabolism, slightly increased brain concentrations of 8-OH-DPAT. Pindolol, which antagonized the elevation of serum corticosterone concentration by 8-OH-DPAT, did not alter brain concentrations of 8-OH-DPAT. The analytical method should be useful in correlating brain concentrations of 8-OH-DPAT with various neurochemical, behavioral or other functional effects that have been described for this compound.

8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) was first described as a serotonin agonist by Arvidsson *et al.* [1]. It was later shown to have selective affinity for the 5HT-1A subtype of serotonin receptors [2]. 8-OH-DPAT produces various effects in rats such as decreased brain serotonin turnover [3], altered sexual behavior [4], increased food intake [5], increased serum corticosterone concentration [6], components of the serotonin behavioral syndrome [7], anti-immobility activity in a forced swimming test [8] and hypothermia [9]. 8-OH-DPAT is much more potent in producing these effects when given subcutaneously than when given intraperitoneally [10] or orally [Fuller and Snoddy, unpublished observations], suggesting that 8-OH-DPAT may be subject to rapid first-pass metabolism. The effects of 8-OH-DPAT are relatively short in duration [3, 9], also consistent with rapid metabolism of the drug. However, no data on drug concentrations in blood or tissues have been published. We are describing here a liquid chromatographic method using electrochemical detection for the measurement of 8-OH-DPAT concentrations in brain and data generated by using this method on the brain concentrations of 8-OH-DPAT in rats as a function of time after drug administration, dose level, and route of administration.

METHODS

Male Sprague–Dawley rats weighing 150–200 g were purchased from Charles River Breeding Laboratories (Portage, MI). 8-OH-DPAT, synthesized in the Lilly Research Laboratories by Robert D. Titus or obtained from Research Biochemicals Inc. (Natick, MA), was injected subcutaneously or intraperitoneally in aqueous solution. Rats were decapitated, and whole brains were quickly removed, frozen on dry ice, and stored at −15°. 5-Hydroxyindoleacetic acid (5-HIAA) concentrations were measured by liquid chromatography with electrochemical detection as described previously [11]. Serum corticosterone was measured spectrofluorometrically [12].

The procedures used for the assay of 8-OH-DPAT are as follows. The analytical column was an Econosphere 5 μm CN, 4.6 × 150 mm (Alltech Associates). The mobile phase was 0.05 M NaH_2PO_4:CH_3OH:CH_3CN (40:30:30) at a flow rate of 1 ml/min, and a temperature of 23°. Detection of 8-OH-DPAT for all data reported in this paper was accomplished electrochemically with a Coulochem model 5100A (ESA, Bedford, MA). Both the model 5011 cell and the model 5010 cell were used with electrode 1 (screening electrode) at 0.4 V and electrode 2 (detecting electrode) at 0.75 V. The model 5011 cell with a small surface area detection electrode was subject to fatigue (loss of sensitivity); however, a new model 5010 cell showed no fatigue, presumably because of its larger surface area and total oxidation of the compound. A Bioanalytical Systems (Lafayette, IN) LC4B electrochemical detector (with a glassy carbon electrode at a potential of 0.9 V) was also tested and found to be suitable for the assay of 8-OH-DPAT.

* Author to whom correspondence should be addressed.

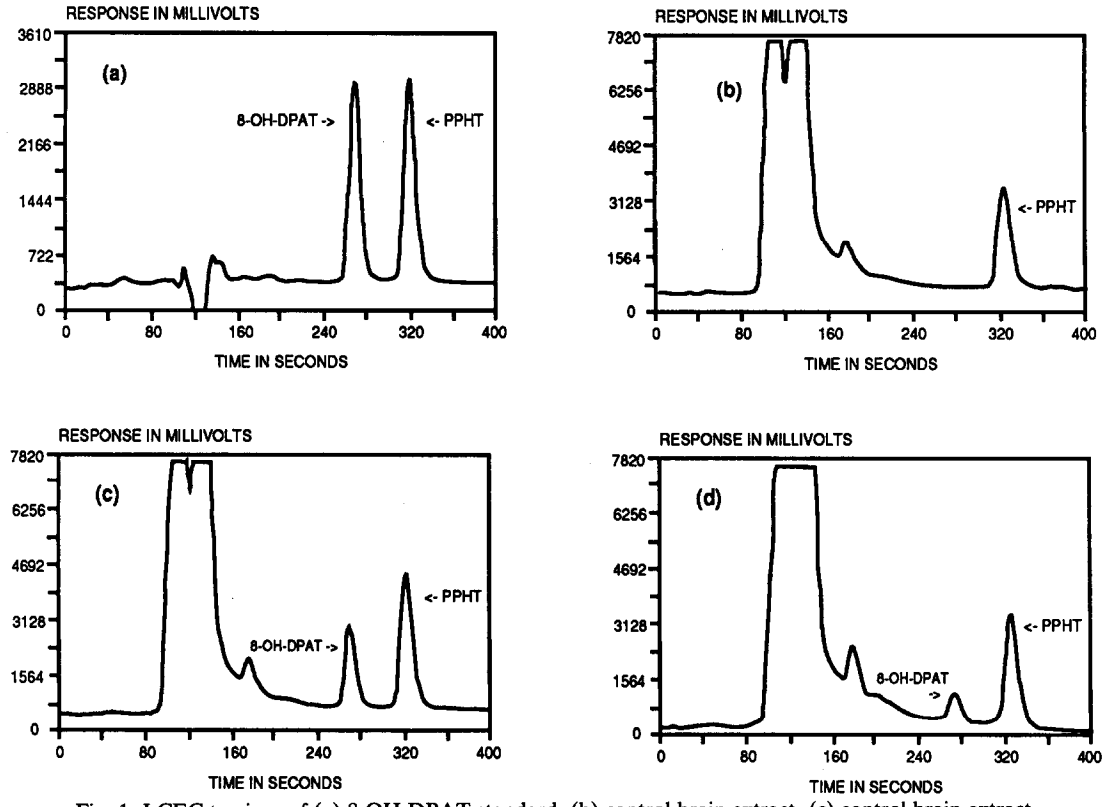

Fig. 1. LCEC tracings of (a) 8-OH-DPAT standard, (b) control brain extract, (c) control brain extract to which 8-OH-DPAT was added, and (d) brain extract from rat treated with 8-OH-DPAT. The internal standard PPHT was added to all samples.

Brain tissue was sonicated in 10 vol. of 0.1 N trichloroacetic acid (TCA) which contained 250 ng/ml 5-hydroxy-2-(-phenylethyl-*N*-*n*-propylamino)tetralin HCl (PPHT) (Research Biochemicals Inc.) as an internal standard and centrifuged at 12,000 *g*. Solid-phase extraction columns (Analytichem C18 Bond-Elut) were used to isolate the 8-OH-DPAT as follows: 1 ml of TCA supernatant fluid was aspirated through the column and then successive rinses of 1 ml of distilled water, 750 μl of mobile phase:water (1:2). 8-OH-DPAT was then eluted with 500 μl of mobile phase containing an additional 10% acetonitrile. The organic solvents were evaporated by heating at 60° for about 30 min to concentrate the samples to a volume of 300 μl, although this was not always necessary. Then 30–50 μl was injected onto the analytical column with a WISP autoinjector (Waters Associates).

In some experiments a Varian AASP was used to inject the sample directly onto the analytical column. This had advantages of convenience, speed and sensitivity. One milliliter of the TCA supernatant fluid was applied to a C8 solid phase extraction cassette designed for the AASP (Analytichem), and the samples were washed through the cassette with 1 ml of water. The cassettes were placed in holders on the AASP and rinsed with a purge cycle. The purge solvent was mobile phase buffer:water (1:3). The pre-inject purge was set at 10 and the post-inject purge set at 5. The entire sample was then eluted directly onto the analytical column with the mobile phase.

With the C18 Bond-Elut columns, overall recoveries were determined in ten 1-ml samples of TCA homogenate to which had been added 100 ng of 8-OH-DPAT and 250 ng of PPHT. Recovery for 8-OH-DPAT averaged 74% with a coefficient of variation (CV) of 6.3%, and recovery for PPHT averaged 40% with a CV of 7.5%. The CV of the ratio 8-OH-DPAT/PPHT was 4.06%. In other experiments, recoveries of 8-OH-DPAT and PPHT from TCA alone were 85 and 95% respectively. Apparently PPHT binds to tissue to a much greater extent than does 8-OH-DPAT—probably because of its more hydrophobic structure—but it is still useful as an internal standard since it decreases the CV.

There are three important criteria for an internal standard in this assay: (1) it should contain the same type of electroactive functional group as 8-OH-DPAT (i.e. a hydroxy-aminotetralin); (2) it must be chromatographically different from 8-OH-DPAT; and (3) it should be readily available. The only compound which was found to meet all of these criteria was PPHT. The CV for this assay was low even without the internal standard, and the assay could be done without an internal standard. Nevertheless, the use of PPHT as an internal standard is recommended in order to correct for possible electrode fatigue, sample transfer differences, and detector scale changes, and to serve as an indicator

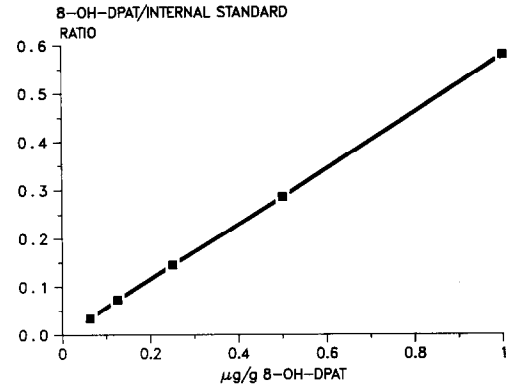

Fig. 2. Standard curve for 8-OH-DPAT. A normal rat brain was homogenized in 10 vol. of trichloroacetic acid containing 250 ng/ml of the internal standard PPHT. 8-OH-DPAT standards were added to 1-ml aliquots of the homogenate.

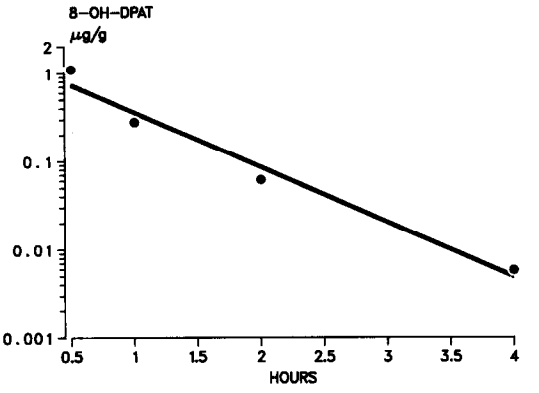

Fig. 3. Decline in 8-OH-DPAT concentrations in whole brain after injection of a 1 mg/kg s.c. dose of 8-OH-DPAT into rats. Mean values for five rats per group are shown (standard errors bars are hidden by the data points).

if there are any problems with the assay, PPHT cannot be used to correct for recovery of the 8-OH-DPAT from tissue since the recoveries are very different, as noted above. Therefore, standards were run in each assay by adding various amounts of 8-OH-DPAT to a TCA homogenate (30–150 ng 8-OH-DPAT/ml of 10% homogenate) containing PPHT (250 ng/mg 0.1 N TCA). Standard curves were generated by a Hewlett–Packard 1000 chromatography data system computer based on peak height ratios. The computer also calculated sample concentrations in the experiments where this was necessary.

RESULTS

Figure 1 shows the retention time of 8-OH-DPAT to be about 270 sec and that for the internal standard PPHT to be about 320 sec in the chromatographic system used (Fig. 1a), and no electrochemically active material from brain extracts of control rats was present in this region of the chromatogram (Fig. 1b). The presence of 8-OH-DPAT in an extract from rats treated with 8-OH-DPAT (1 mg/kg s.c., 1 hr before the animals were killed) (Fig. 1d) coincided well with 8-OH-DPAT added to a brain extract from control rats (Fig. 1c). The standard curve for 8-OH-DPAT (Fig. 2) shows linearity of the electrochemical response over a concentration range of 8-OH-DPAT added to brain extracts equivalent to 0.06 to 1.0 μg/g tissue. The lower limit of detection of 8-OH-DPAT determined from other experiments would be approximately 5 ng/g brain.

The presence of 8-OH-DPAT in whole brain after injection of 8-OH-DPAT into rats, and the rate of disappearance of 8-OH-DPAT from brain are shown in Fig. 3. The brain concentration of 8-OH-DPAT declined from >1.0 μg/g at 30 min to less than 10 ng/g at 4 hr. The half-life of 8-OH-DPAT in brain calculated from these data was 26 min.

The concentrations of 8-OH-DPAT in rat brain after administration of 8-OH-DPAT via different routes are shown in Fig. 4. When 8-OH-DPAT was given intraperitoneally, the doses had to be about 10-fold higher than subcutaneous doses to produce equivalent brain concentrations of 8-OH-DPAT.

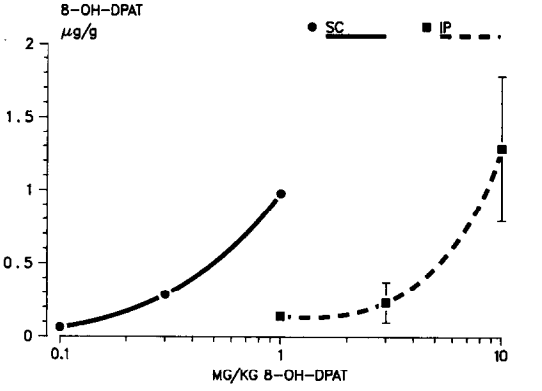

Fig. 4. Concentrations of 8-OH-DPAT in whole brain after s.c. or i.p. injection of 8-OH-DPAT into rats. Mean value ± SE for five rats per group are shown.

Table 1. Concentrations of 8-OH-DPAT in various brain regions

Brain region	8-OH-DPAT, (μg/g)
Hippocampus	0.68 ± 0.04
Hypothalamus	0.68 ± 0.04
Striatum	0.62 ± 0.06
Cerebellum	0.62 ± 0.05
Brain stem	0.61 ± 0.04
Midbrain	0.46 ± 0.04

8-OH-DPAT was injected at 1 mg/kg s.c. 30 min before the rats were killed. Mean values ± SE for five rats per group are shown.

Table 1 shows the concentrations of 8-OH-DPAT in six different regions of rat brain at 30 min after its injection. Except for a slightly lower concentration of 8-OH-DPAT in the midbrain in this experiment,

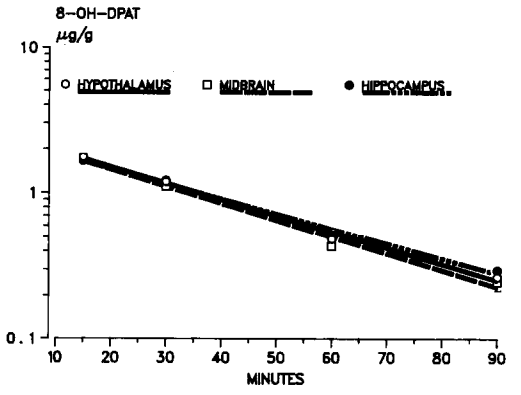

Fig. 5. 8-OH-DPAT concentrations and disappearance from three regions of rat brain (hypothalamus, midbrain and hippocampus) after a 1 mg/kg s.c. dose. Mean values for five rats per group are shown (standard error bars are hidden by the data points).

concentrations varied little among the regions studied. In a separate experiment, 8-OH-DPAT concentrations were measured in midbrain, hypothalamus and hippocampus at different times after 8-OH-DPAT injection (Fig. 5). In this experiment the concentrations and rates of disappearance of 8-OH-DPAT were identical among all the three brain regions.

Table 2 shows that proadifen (SKF-525A), an inhibitor of drug metabolism, slightly increased the concentration of 8-OH-DPAT in rat brain. A statistically significant, but only 35%, increase in 8-OH-DPAT concentrations was produced by SKF-525A pretreatment when 8-OH-DPAT was injected s.c. A slightly greater increase (88%) in 8-OH-DPAT concentrations was produced when 8-OH-DPAT was injected i.p., but the larger within-group variation after this route of administration prevented the effect of SKF-525A from being statistically significant.

Table 3 shows the effect of pretreatment with (±)pindolol on a 5HT-1A-receptor-mediated effect of 8-OH-DPAT, namely an increase in serum corticosterone concentration. 8-OH-DPAT caused a 12-fold increase in serum corticosterone concentration. Pindolol pretreatment significantly attenuated but did not prevent completely that increase, while the concentration of 8-OH-DPAT in brain was not changed.

DISCUSSION

The method currently described provides a sensitive and convenient means of measuring 8-OH-DPAT concentrations in brain, permitting their correlation with various neurochemical, behavioral, endocrinologic and other effects that have been observed with 8-OH-DPAT. Previously no data on 8-OH-DPAT levels in tissues have been reported. Our findings show that brain concentrations of 8-OH-DPAT are easily measurable in whole brain or in brain regions after s.c. doses of 8-OH-DPAT at least as low as 0.1 mg/kg. The assay was first designed for the AASP, and conditions for utilizing the AASP are given to aid laboratories which have one. For laboratories not having an AASP, conditions are also given for using the standard Bond-Elut columns which are in wide use.

The major influence of route of administration of

Table 2. Effect of proadifen pretreatment on brain concentrations of 8-OH-DPAT

Dose of 8-OH-DPAT	8-OH-DPAT concentration in whole brain (μg/g)	
	No pretreatment	Proadifen pretreatment
1 mg/kg s.c.	1.17 ± 0.06	1.57 ± 0.12*
3 mg/kg i.p.	0.58 ± 0.13	1.09 ± 0.33

8-OH-DPAT was injected 30 min before the rats were killed and 1 hr after proadifen (50 mg/kg i.p.). Mean values ± SE for five rats per group are shown.
* Significant difference from group without pretreatment ($P < 0.05$).

Table 3. Effect of pindolol pretreatment on serum corticosterone elevation by 8-OH-DPAT and on hypothalamus concentrations of 8-OH-DPAT in rats

Dose of 8-OH-DPAT (mg/kg s.c.)	Serum corticosterone (μg/100 ml)		8-OH-DPAT concentration in hypothalamus (μg/g)
	Vehicle	8-OH-DPAT	60 min after 8-OH-DPAT
No pretreatment	3.4 ± 0.6	43.8 ± 2.5*	0.29 ± 0.03
Pindolol pretreatment	6.2 ± 0.8	24.0 ± 4.7*†	0.33 ± 0.03

8-OH-DPAT (1 mg/kg s.c.) was injected 60 min before the rats were killed and 15 min after (±)pindolol. Mean values ± SE for five rats per group are shown.
* Significant increase in corticosterone induced by 8-OH-DPAT ($P < 0.05$).
† Significant attenuation of corticosterone increase by pindolol pretreatment ($P < 0.05$).

8-OH-DPAT on brain concentrations of the drug correlates well with the importance of route of administration as a determinant of 8-OH-DPAT potency in decreasing brain serotonin turnover [10]. Our current findings show that the dose of 8-OH-DPAT had to be approximately ten times higher when it was given intraperitoneally than when given subcutaneously to produce equivalent brain levels. Earlier we had reported that 8-OH-DPAT was at least ten times more potent in decreasing brain serotonin turnover when it was given subcutaneously compared to intraperitoneally. Probably a rapid first-pass metabolism in liver accounts for the large effect of route of administration. Such metabolism may also explain the rapid disappearance of 8-OH-DPAT from brain (half-life = 26 min).

One application of the method for determining 8-OH-DPAT is seen in the experiment described in Table 3. (±)Pindolol antagonized the increase in serum corticosterone concentration elicited by 8-OH-DPAT. Since this increase is thought to be initiated by activation of 5HT-1A receptors in brain, one mechanism by which the increase could be antagonized is by inhibition of 8-OH-DPAT entry into the brain. However, brain concentrations of 8-OH-DPAT were found to be unaffected by (±)pindolol. That finding, together with the known affinity of (±)pindolol for 5HT-1A receptors [13], supports the interpretation that (±)pindolol attenuated the elevation of serum corticosterone concentration by 8-OH-DPAT through blockade of central 5HT-1A receptors.

Acknowledgement—We thank Harold D. Snoddy for participation in the experiment in which corticosterone concentration was measured.

REFERENCES

1. Arvidsson L-E, Hacksell U, Nilsson JLG, Hjorth S, Carlsson A, Lindberg P, Sanchez D and Wikstrom H, 8-Hydroxy-2-(*n*-propylamino)tetralin, a new centrally acting 5-hydroxytryptamine receptor agonist. *J Med Chem* **24**: 921–923, 1981.
2. Middlemiss DN and Fozard JR, 8-Hydroxy-2-(di-*n*-propylamino)tetralin discriminates between subtypes of the 5-HT_1 recognition site. *Eur J Pharmacol* **90**: 151–153, 1983.
3. Hjorth S, Carlsson A, Lindberg P, Sanchez D, Wikstrom H, Arvidsson L-E, Hacksell U and Nilsson JLG, 8-Hydroxy-2-(di-*n*-propylamino)tetralin, 8-OH-DPAT, a potent and selective simplified ergot congener with central 5-HT-receptor stimulating activity. *J Neural Transm* **55**: 169–188, 1982.
4. Ahlenius S, Larsson K, Svensson L, Hjorth S, Carlsson A, Lindberg P, Wikstrom H, Sanchez D, Arvidsson L-E, Hacksell U and Nilsson JLG, Effects of a new type of 5-HT receptor agonist on male rat sexual behavior. *Pharmacol Biochem Behav* **15**: 785–792, 1981.
5. Dourish CT, Hutson PH and Curzon G, Low doses of the putative serotonin agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) elicit feeding in the rat. *Psychopharmacology* **86**: 197–204, 1985.
6. Koenig JI, Gudelsky GA and Meltzer HY, Stimulation of corticosterone and β-endorphin secretion in the rat by selective 5-HT receptor subtype activation. *Eur J Pharmacol* **137**: 1–8, 1987.
7. Tricklebank MD, Forler C and Fozard JR, The involvement of subtypes of the 5-HT_1 receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-*n*-propylamino)tetralin in the rat. *Eur J Pharmacol* **106**: 271–282, 1984.
8. Cervo L and Samanin R, Potential antidepressant properties of 8-hydroxy-2-(di-*n*-propylamino)tetralin, a selective serotonin$_{1A}$ receptor agonist. *Eur J Pharmacol* **144**: 223–229, 1987.
9. Hjorth S, Hypothermia in the rat induced by the potent serotonergic agent 8-OH-DPAT. *J Neural Transm* **61**: 131–135, 1982.
10. Fuller RW and Snoddy HD, Influence of route of administration on potency of the selective 5HT-1A agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin, in rats. *Res Commun Chem Pathol Pharmacol* **58**: 409–412, 1987.
11. Fuller RW, Snoddy HD and Perry KW, *p*-Chloroamphetamine formation responsible for long-term depletion of brain serotonin after *N*-cyclopropyl-*p*-chloroamphetamine injection in rats. *Life Sci* **40**: 1921–1927, 1987.
12. Solem JH and Brinck-Johnsen T, An evaluation of a method for determination of free corticosteroids in minute quantities of mouse plasma. *Scand J Clin Lab Invest* **17** (Suppl. 80): 1–14, 1965.
13. Hamon M, Cossery J-M, Spampinato U and Gozlan H, Are there selective ligands for 5-HT1A and 5-HT1B receptor binding sites in brain? *Trends Pharmacol Sci* **7**: 336–338, 1986.