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Discriminative Stimulus Properties of the 5-HT_{1A} Agonist 8-Hydroxy-2-(Di-*n*-Propylamino)Tetralin (8-OH DPAT)

RICHARD A. GLENNON

Department of Medicinal Chemistry, School of Pharmacy, Medical College of Virginia Virginia Commonwealth University, Richmond, VA 23298

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GLENNON, R. A. *Discriminative stimulus properties of the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH DPAT).* PHARMACOL BIOCHEM BEHAV **25**(1) 135–139, 1986.—Using a two-lever operant procedure, eleven rats were trained to discriminate 0.2 mg/kg of the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH DPAT) from saline using a variable-interval 15 sec schedule of reinforcement. Once trained, these animals were used in a series of stimulus generalization and stimulus antagonism studies. The 8-OH DPAT-stimulus did not generalize to the 5-HT_{1B} agonist 1-(3-trifluoromethylphenyl)piperazine (TFMPP) or the 5-HT_2 agonist 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM), nor could it be attenuated by pre-treatment of the animals with the 5-HT_2 antagonist ketanserin. Low doses of spiperone and propranolol were without effect on 8-OH DPAT-appropriate responding, whereas higher doses of these agents resulted in disruption of behavior. Some preliminary structure-activity data were also obtained using several related tetralin analogs. The results of this study demonstrate that the serotonin agonist 8-OH DPAT serves as a discriminative stimulus in rats and that it produces stimulus effects that are probably not 5-HT_{1B} or 5-HT_2-mediated.

8-OH DPAT TFMPP DOM 5-HT_1 5-HT_2 5-HT_{1A} 5-HT_{1B} Ketanserin
Drug discrimination

TWO major populations of central serotonin (5-HT) binding sites have been defined on the basis of radioligand binding data; these sites have been termed 5-HT_1 and 5-HT_2 [11,16]. [^{3}H]Spiperone can differentiate between two subpopulations of 5-HT_1 sites: this ligand binds with high affinity to 5-HT_{1A} sites and possesses about a 3000-fold lower affinity for 5-HT_{1B} sites [15]. Recently, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH DPAT) has been demonstrated to be a 5-HT_{1A}-selective agonist [13], and [^{3}H]8-OH DPAT has been introduced as a radioligand for selectively labeling central 5-HT_{1A} sites [8,10].

We have previously shown that the 5-HT_2 agonists 1-(2,5-dimethoxy-4X-phenyl)-2-aminopropane, where X= methyl and iodo (i.e., DOM and DOI, respectively), can serve as discriminative stimuli in animals [4–6]. Stimulus generalization occurs with other putative 5-HT_2 agonists but not with 5-HT_1 agonists such as 8-OH DPAT and 1-(3-trifluoromethylphenyl) piperazine (TFMPP) [5]. TFMPP, a 5-HT_{1B} agonist [17], also serves as a discriminative stimulus; TFMPP-stimulus generalization occurs with other 5-HT_{1B} agonists, but not with 5-HT_{1A} or 5-HT_2 agonists such as 8-OH DPAT or DOM, respectively [7,12]. In order to better understand the pharmacological/behavioral properties of 5-HT_{1A} agonists, we initiated a drug discrimination study using 8-OH DPAT as the training drug. A preliminary account of this work has been presented [5].

METHOD

Subjects/Apparatus

The animals used in this study were eleven male (225–300 g) Sprague-Dawley rats. Initially, six subjects were trained to discriminate 8-OH DPAT from saline; subsequently, an additional five animals were trained and added to the group. The animals were housed individually and were maintained at approximately 80% of their free-feeding body weights; drinking water was always available in the home cages. Behavioral testing was conducted in standard two-lever operant chambers (Model E 10-10, Coulbourn Instruments) housed within light- and sound-attenuating outer chambers. Illumination of each chamber was provided by an overhead 28 V houselight. Solid state and electromechanical programming and recording equipment were used and were housed in the same room as the operant chambers.

Discrimination Procedure

Briefly, all rats were trained to respond on both levers for sweetened milk reward under a variable interval 15-sec (VI-

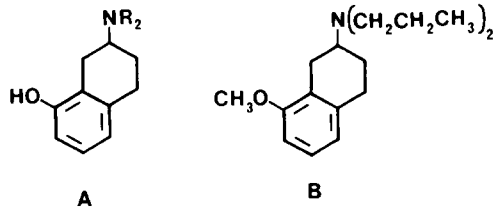

FIG. 1. Structures of aminotetralin analogs used in this study: 8-OH DPAT (A, R=$CH_2CH_2CH_3$), 8-OH DEAT (A, R=CH_2CH_3), 8-OH DBAT (A, R=$CH_2CH_2CH_2CH_3$), 8-OMe DPAT (B).

15) schedule of reinforcement. After lever-responding was established, each daily session was preceded by an intraperitoneal (IP) injection of either racemic 8-OH DPAT (0.2 mg/kg) or sterile 0.9% saline (1.0 ml/kg). A pre-session injection interval of 15 min was employed; immediately following administration of 8-OH DPAT or saline, the animals were returned to their home cages until the designated time had elapsed. Training sessions were of 15 min duration. For approximately half of the animals, responding on right lever was reinforced after administration of 8-OH DPAT, whereas responding on the opposite lever was reinforced after administration of saline. The situation was reversed for other half of the animals. Saline or 8-OH DPAT was administered on a double-alternation schedule (i.e., two days saline, two days drug) six days per week. On every fifth day (i.e., one block of sessions) discrimination learning was assessed during an initial 2.5-min non-reinforced (extinction) session, followed by a 12.5-min training session. Data collected during the extinction session included total responses (expressed as mean responses per min) and distribution of responses (expressed as percent of total responses on the 8-OH DPAT-appropriate lever). After discrimination performance was stable under each treatment condition (i.e., after about twenty-five blocks of sessions; see Fig. 2), the stimulus generalization and stimulus antagonism studies were begun.

Stimulus Generalization Studies

Maintenance of the 8-OH DPAT/saline discrimination was insured in all eleven animals by continuation of the training sessions throughout the remainder of the studies. Discrimination training sessions were conducted with 8-OH DPAT or saline during the two days prior to any generalization test. Discrimination training was assessed (not more than once every three days) during a 2.5-min extinction session (followed by a 12.5-min training session). Animals not discriminating drug from saline (i.e., animals making less than 80% of their responses on the 8-OH DPAT-appropriate lever after administration of the training dose of 8-OH DPAT, or making more than 20% of their responses on the same lever after administration of saline) were not used in the immediately subsequent stimulus generalization test session. During investigations of stimulus generalization, test sessions (in which one dose of a given agent was evaluated) were interposed amongst the training sessions and were separated by an odd (not less than three) number of days. The animals were allowed 2.5 min to respond under non-reinforcement conditions and were then returned to their home cages. The generalization tests investigated the ability of the training drug stimulus to generalize to other

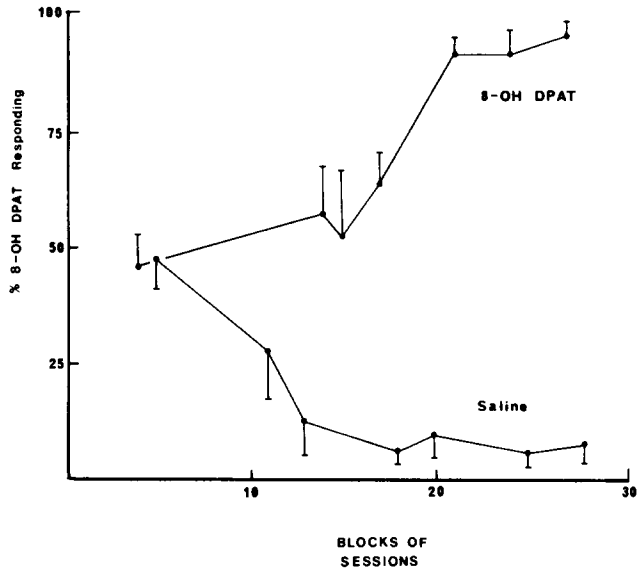

FIG. 2. Learning curve for the acquisition of the 8-OH DPAT (0.2 mg/kg) vs. saline (1.0 ml/kg) discrimination by the original group of six animals. (Blocks represent the number of double-alternation training periods.)

doses of the training drug, or to doses of other agents. Doses of these agents were administered in a random sequence to groups of, routinely, 3 to 5 animals such that a dose of more than one agent could be evaluated during a given week. No animal received the same dose of a given agent more than once. A 15-min pre-session injection interval was used throughout. Stimulus generalization was defined in this study as being 80% or greater 8-OH DPAT-appropriate responding. That is, stimulus generalization was said to have occurred when the animals, after being given a dose of challenge drug, made 80% or greater of their total responses on the drug-appropriate lever. Animals making less than five total responses during the entire 2.5-min extinction session were reported as being disrupted. Where generalization occurred, an ED50 value was determined by the method of Finney [3]. These ED50 values are doses at which the animals would be anticipated to make 50% of their responses on the drug-appropriate lever.

Stimulus Antagonism Studies

In essence, these studies were conducted in a manner similar to those above except that doses of the antagonist were administered 15 min (propranolol), 45 min (ketanserin), or 60 min (spiperone) prior to the administration of either the training dose of 8-OH DPAT or, in the control studies, to 1.0 ml/kg of saline. Fifteen min later, the animals were placed in the operant chambers for a 2.5-min non-reinforced test session.

Drugs

Racemic 8-hydroxy-2-(di-*n*-propylamino) tetralin hydrobromide (8-OH DPAT) and spiperone were purchased from Research Biochemicals Inc. (Wayland, MA), and racemic propranolol hydrochloride from Sigma (St. Louis, MO). The following agents were obtained as gifts: 8-hydroxy-2-(di-

TABLE 1

RESULTS OF STIMULUS GENERALIZATION STUDIES USING 8-OH DPAT (0.2 mg/kg) AS THE TRAINING DRUG*

Agent	Dose (mg/kg)	N†	8-OH DPAT Appropriate Responding‡ (±SEM)	Mean Resp Per Min‡ (±SEM)
8-OH DPAT	0.2	11/11	96%(± 2)	27.8(± 6.4)
Saline (1 ml/kg)		11/11	9%(± 2)	31.2(± 5.1)
8-OH DPAT#	0.05	3/3	32%(±13)	32.0(± 2.7)
	0.10	4/4	46%(±13)	34.3(± 6.1)
	0.14	5/5	87%(± 6)	21.8(± 5.0)
	0.20	5/5	95%(± 2)	25.7(± 3.4)
	ED50=0.08 (0.04–0.13) mg/kg§			
TFMPP	0.1	3/3	5%(± 3)	28.0(± 7.2)
	0.3	2/3	21%(± 9)	5.4(± 1.4)
	0.5	3/5	34%(±17)	2.4(± 0.2)
	0.6	0/3	—¶	
DOM	0.2	3/3	7%(± 5)	37.0(±11.1)
	0.4	2/2	23%(± 6)	38.6(±14.2)
	1.0	3/3	7%(± 2)	38.4(± 8.1)
	3.0	3/3	15%(± 6)	8.4(± 0.8)
	3.5	2/4	10%(± 9)	3.6(± 0.4)
	4.0	2/5	—¶	
5-OMe DMT	0.1	3/3	2%(± 1)	27.7(± 5.3)
	0.3	3/3	26%(±16)	4.4(± 1.3)
	0.5	3/4	20%(± 9)	8.5(± 3.0)
	0.7	2/3	38%(± 3)	7.2(± 2.4)
	0.75	1/3	—¶	—
	0.80	0/3	—	—
	1.0	0/3	—	—
Fenfluramine	1.5	3/3	21%(±14)	24.0(±10.4)
	2.5	3/4	3%(± 2)	17.2(± 2.7)
	3.0	4/4	8%(± 7)	15.8(± 6.6)
	6.0	3/4	11%(± 7)	12.4(± 8.8)
	8.0	2/3	24%(± 9)	4.6(± 1.0)
	8.2	2/8	—¶	—
	8.5	1/8	—	—
8-OH DEAT	0.10	4/4	21%(± 6)	36.3(± 7.8)
	0.15	4/4	60%(±16)	25.0(± 6.0)
	0.20	3/3	91%(± 3)	21.2(± 0.8)
	ED50=0.13 (0.08–0.19) mg/kg			
8-OMe DPAT	0.15	3/3	18%(± 7)	20.4(±10.5)
	0.2	3/3	41%(±14)	17.7(± 6.3)
	0.4	3/4	99%(± 1)	17.8(± 5.8)
	ED50=0.22 (0.14–0.30) mg/kg			
8-OH DBAT	0.2	4/4	10%(± 9)	18.6(± 1.0)
	0.5	3/3	3%(± 1)	32.0(±11.4)
	2.0	3/3	4%(± 3)	34.4(±11.7)
	4.0	4/4	6%(± 2)	26.4(± 4.7)

*A 15-min pre-session injection interval was used throughout.
†Number of animals responding/number to receive drug.
‡Data obtained during 2.5-min extinction session.
§ED50 values followed by 95% confidence limits.
¶Disruption of behavior (i.e., no responding).
#ED50 values for 8-OH DPAT, 8-OH DEAT, 8-OMe DPAT are 0.24, 0.43 and 0.74 μmoles/kg, respectively.

TABLE 2

RESULTS OF STIMULUS ANTAGONISM STUDIES USING 8-OH DPAT (0.2 mg/kg) AS THE TRAINING DRUG*

Antagonist	Dose (mg/kg)	N†	8-OH DPAT Appropriate Responding‡ (±SEM)	Mean Resp Per Min‡ (±SEM)
Ketanserin	0.2	2/3	90%(± 1)	11.6(± 0.4)
	0.3	3/3	93%(± 4)	11.7(± 5.0)
	0.5	3/3	98%(± 2)	4.3(± 1.1)
Spiperone	0.01	4/4	80%(±20)	6.0(± 3.3)
	0.03	3/4	100%	4.6(± 2.6)
	0.05	1/3	—¶	
	0.10	1/4	—	
Propranolol	0.1	3/3	99%(± 1)	6.1(± 2.1)
	0.3	2/2	100%	6.0(± 3.6)
	0.5	1/4	—¶	
	2.0	1/5	—	
	3.5	1/5	—	

Pre-session injection interval for 8-OH DPAT was 15 min; see the Method section for pre-session injection intervals used for antagonists. See Table 1 for footnotes.

ethylamino)tetralin hydrobromide (8-OH DEAT) and 8-hydroxy-2-(di-*n*-butylamino)tetralin hydrobromide (8-OH DBAT) (Fig. 1) (U. Hacksell and L.-E. Arvidsson, Uppsala University), fenfluramine hydrochloride (A. H. Robins Co., Richmond, VA), and ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium). The remaining compounds had been previously synthesized in our laboratories and include: 5-methoxy-N,N-dimethyltryptamine hydrogen oxalate (5-OMe DMT), 1-(3-trifluoromethylphenyl) piperazine hydrochloride (TFMPP), racemic 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane hydrochloride (DOM), and 8-methoxy-2-(di-*n*-propylamino)tetralin hydrochloride (8-OMe DPAT). All solutions were prepared fresh daily in 0.9% sterile saline. Spiperone was dissolved in one equivalent of 0.1 N hydrochloric acid prior to dilution with saline. Administration of all drugs was via intraperitoneal injection.

RESULTS

After approximately twenty-five training sessions under each drug condition, the animals were able to consistently discriminate 8-OH DPAT (0.2 mg/kg) from saline (Fig. 2). Responding to the training drug was dose related in that administration of lower doses of 8-OH DPAT produced a decrease in 8-OH DPAT-appropriate responding (Table 1). In tests of stimulus generalization, administration of doses of the 5-HT agonists TFMPP, DOM, and 5-methoxy-N,N-dimethyltryptamine (5-OMe DMT) resulted in a maximum of 34%, 10%, and 38%, respectively, 8-OH DPAT-appropriate responding (Table 1). Fenfluramine produced a maximum of 24% 8-OH DPAT-appropriate responding (Table 1). In each case, a small increase in dose above that which produced these effects resulted in disruption of behavior. Two DPAT analogs, that is, 8-OH DEAT and 8-OMe DPAT, produced 8-OH DPAT-appropriate responding and were nearly as potent as 8-OH DPAT itself (Table 1). 8-OH DBAT did not produce 8-OH DPAT-like effects (Table 1).

In tests of stimulus antagonism, neither ketanserin, spiperone, nor propranolol were able to attenuate 8-OH DPAT-appropriate responding at the doses evaluated (Table 2). In the control studies, administration of these agents in combination with saline (data not shown) resulted in 8-OH DPAT-appropriate responding that did not exceed 20% (except for 24% for 0.2 mg/kg of ketanserin). In a preliminary set of studies, fenfluramine was also examined as a potential antagonist. Administration of 0.5 mg/kg of fenfluramine 15 min prior to 0.2 mg/kg of 8-OH DPAT resulted in 85% (N=4/4) 8-OH DPAT-appropriate responding; administration of higher doses of fenfluramine resulted in disruption of behavior.

DISCUSSION

The 5-HT_{1A} agonist 8-OH DPAT, at 0.2 mg/kg, serves as a discriminative stimulus in rats (ED50=0.08 mg/kg) (Table 1). The 8-OH DPAT-stimulus did not generalize to doses of the 5-HT_{1B} agonist TFMPP or the 5-HT_2 agonist DOM (Table 1). Likewise, stimulus generalization did not occur with 5-OMe DMT. This latter agent has been demonstrated to bind both to 5-HT_1 and 5-HT_2 sites [11], and may lack the selectivity necessary for stimulus generalization to occur.

Fenfluramine is an agent that releases endogenous stores of 5-HT [2]; thus, it might be anticipated that this agent would result in stimulus generalization regardless of the selectivity of the training drug. To this extent, both a DOM-stimulus (unpublished data) and a TFMPP-stimulus [12] generalize to this agent. The 8-OH DPAT-stimulus did not, however, generalize to fenfluramine (Table 1). At this point, we cannot explain this lack of stimulus generalization with fenfluramine. Nevertheless, consistent with the present results is the finding by Young (personal communication) that stimulus generalization did not occur between fenfluramine and 8-OH DPAT in fenfluramine-trained animals.

The last series of stimulus generalization studies examined the effect of structural modification on 8-OH DPAT-appropriate responding. Three aminotetralin analogs were examined: 8-OH DEAT, 8-OH DBAT and 8-OMe DPAT (Fig. 1). On a molar basis, 8-OH DEAT, the N,N-diethyl analog of 8-OH DPAT, was nearly half as potent as the training drug, and 8-OMe DPAT, the O-methyl ether of 8-OH DPAT, was approximately one-third as potent as 8-OH DPAT itself. The results obtained for 8-OH DBAT support the suggestion by Arvidsson and co-workers [1] that the 5-HT receptors with which 8-OH DPAT interacts are sensitive to steric bulk in the region corresponding to the terminal amine group and cannot easily accommodate alkyl groups larger than *n*-propyl.

None of the serotonin antagonists examined effectively blocked the 8-OH DPAT stimulus. Ketanserin, a 5-HT_2 antagonist, can attenuate by 50% the discriminative effects of 0.5 mg/kg of the 5-HT_2 agonist DOI, in DOI-trained animals, at a dose of 0.03 mg/kg [4]. As shown in Table 2, fifteen times this dose (i.e., 0.5 mg/kg) had no effect on 8-OH DPAT-appropriate responding. Spiperone, which binds with high affinity both to 5-HT_2 and 5-HT_{1A} binding sites [11,15], had no effect on 8-OH DPAT-appropriate responding at doses of up to 0.03 mg/kg; higher doses resulted in disruption of behavior. The β-adrenergic antagonist propranolol, which displays a high affinity for 5-HT_1 binding sites [14], and which has been shown to behave in some instances as a 5-HT_1 antagonist *in vivo* [9], had no effect on 8-OH DPAT-

appropriate responding (Table 2). Ketanserin was not expected to have an effect on the 8-OH DPAT stimulus; that spiperone and propranolol were without effect is somewhat surprising. However, it should be noted that these agents produced disruption of behavior at fairly low doses, making it impossible to draw conclusions regarding their potential antagonist effects.

In summary, the 5-HT_{1A} agonist 8-OH DPAT produces discriminative stimulus effects in rats that are dissimilar to those of the 5-HT_{1B} agonist TFMPP and the 5-HT_2 agonist DOM. This finding is consistent with our earlier reports of a lack of stimulus generalization with 8-OH DPAT using TFMPP-trained and DOM-trained animals. Furthermore, the 8-OH DPAT stimulus could not be antagonized by pretreatment of the animals with the 5-HT_2 antagonist ketanserin. The results of preliminary structure-activity studies using three 8-OH DPAT analogs agree with the results of previously reported *in vitro* studies [1]. Animals trained to discriminate 8-OH DPAT from saline may be useful for the evaluation of novel 5-HT_{1A} agonists and potential 5-HT_{1A} antagonists.

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REFERENCES

1. Arvidsson, L.-E., U. Hacksell, A. M. Johansson, L. G. Nilsson, P. Lindberg, D. Sanchez, H. Wikstrom, K. Svensson, S. Hjorth and A. Carlsson. 8-Hydroxy-2-(alkylamino)tetralins and related compounds as central 5-hydroxytryptamine receptor agonists. *J Med Chem* **27**: 45–51, 1984.
2. Curruba, M., G. B. Picotti, F. Zambotti and P. Mantegazza. Effect of mazindol, fenfluramine and chlorimipramine on 5-hydroxytryptamine uptake and storage mechanisms in rat brain: Similarities and differences. *Naunyn Schmiedebergs Arch Pharmacol* **300**: 227–232, 1977.
3. Finney, D. J. *Probit Analysis*. London: Cambridge University Press, 1952.
4. Glennon, R. A. Discriminative stimulus properties of the serotonergic agent 1-(2,5-dimethoxy-4-iodophenyl)-2-amino propane (DOI). *Life Sci*, in press.
5. Glennon, R. A. and J. D. McKenney. Site-selective serotonin agonists as discriminative stimuli. *Pharmacologist* **27**: 194, 1985.
6. Glennon, R. A., R. Young and J. A. Rosecrans. Antagonism of the effects of the hallucinogen DOM and the purported 5-HT agonist quipazine by 5-HT_2 antagonists. *Eur J Pharmacol* **91**: 189–196, 1983.
7. Glennon, R. A., J. D. McKenney and R. Young. Discriminative stimulus properties of the serotonin agonist 1-(3-trifluoromethylphenyl)piperazine (TFMPP). *Life Sci* **35**: 1475–1480, 1984.
8. Gozlan, H., S. El Mestikawy, L. Pichat, J. Glowinski and M. Hamon. Identification of presynaptic serotonin autoreceptors using a new ligand: ^{3}H-PAT. *Nature* **305**: 140–142, 1983.
9. Green, A. R. and D. J. Heal. The effects of drugs on serotonin-mediated behavioral models. In: *Neuropharmacology of Serotonin*, edited by A. R. Green. Oxford: Oxford University Press, 1985, pp. 326–365.
10. Hamon, M., H. Gozlan, M. D. Hall, S. El Mestikawy, M. B. Emerit and L. Pichat. Identification and properties of presynaptic 5-HT autoreceptors specifically labelled by ^{3}H-PAT in rat CNS. In: *Regulation of Transmitter Function*, edited by E. S. Vizi and K. Magyar. Amsterdam: Elsevier Science Publishers, 1984, pp. 141–151.
11. Leysen, J. E. Characterization of serotonin receptor binding sites. In: *Neuropharmacology of Serotonin*, edited by A. R. Green. Oxford: Oxford University Press, 1985, pp. 79–116.
12. McKenney, J. D. and R. A. Glennon. TFMPP may produce its stimulus effects via a 5-HT_{1B} mechanism. *Pharmacol Biochem Behav* **24**: 43–47, 1986.
13. Middlemiss, D. N. and J. R. Fozard. 8-Hydroxy-2-(di-*n*-propylamino)tetralin discriminates between subtypes of 5-HT_1 recognition sites. *Eur J Pharmacol* **90**: 151–153, 1983.
14. Nahorski, S. R. and A. L. Willcocks. Interaction of β-adrenoceptor antagonists with 5-hydroxytryptamine receptor subtypes in rat cerebral cortex. *Br J Pharmacol* **78**: 107P, 1983.
15. Nelson, D. L., N. W. Pedigo and H. I. Yamamura. Multiple ^{3}H-5-hydroxytryptamine binding sites in rat brain. *J Physiol (Paris)* **77**: 369–372, 1980.
16. Peroutka, S. J. and S. H. Snyder. Multiple serotonin receptors: Differential binding of [^{3}H]5-hydroxytryptamine, [^{3}H]lysergic acid diethylamide and [^{3}H]spiroperidol. *Mol Pharmacol* **16**: 687–699, 1979.
17. Sills, M. A., B. B. Wolfe and A. Frazer. Determination of selective and non-selective compounds for the 5-HT_{1A} and 5-HT_{1B} receptor subtypes in rat frontal cortex. *J Pharmacol Exp Ther* **231**: 480–487, 1984.