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EFFECTS OF TRAZODONE ON SEROTONIN IN THE BRAIN AND PLATELETS OF THE RAT

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Abstract– Trazodone, a novel antidepressant with a structure different from the tricyclic compounds, inhibits the release of brain 5-HT induced by fenfluramine and blocks the uptake of 5-HT by rat platelets. Two known metabolites of trazodone, oxatriazolepyridin propionic acid and trazodone-*N*-oxide, do not share the properties of trazodone. A suggested but unproven metabolite of trazodone, *m*-chlorophenyl piperazine, is however more active than trazodone as an inhibitor of 5-HT uptake. Trazodone and its derivatives are devoid of activity as releasers of 5-HT from rat platelets.

Trazodone, 2-{3-[4(*m*-chlorophenyl)1-piperazinyl]-propyl}-*s*-triazolo [4,3-a]-pirydin-3-(2H)one, is a new psychoactive drug [1–4] with a spectrum of pharmacological activities different from that shown by any known drug acting on the central nervous system [5,6].

It has also been reported that trazodone possesses a peculiar neurochemical profile with regard to its action on brain monoamines [7]. Particular attention has been devoted to the effect of trazodone on brain serotonin and it was suggested that this effect could be of some importance for the clinical, particularly antidepressant, effects of this drug [7–9]. The tricyclic antidepressants, mainly the tertiary amines, are potent blockers of serotonin (5-HT) neuronal uptake mechanism [10–12] and it was recently shown that these drugs prevent the depletion of brain serotonin induced by 4-methyl-ethyl-*m*-tyramine (H 75/12) [10] or by fenfluramine in the rat [13]. This effect was attributed to a blockade by thymoleptics of the accumulation of H 75/12 or fenfluramine into the serotonergic neurons.

In the present experiments the effect of trazodone on the depletion of brain 5-HT induced by fenfluramine in rats was studied in an attempt to investigate further the possible interaction between trazodone and brain serotonin. The effect of trazodone on the uptake and release of [^{14}C]5-HT was also studied in blood platelets, which are considered to be a useful pharmacological model for serotonergic neurons [14–17]. Finally, the effects of three proposed metabolites of trazodone, *m*-chlorophenylpiperazine (CPP), oxotriazolepyridinpropionic acid (OTPA) and the *N*-oxide of trazodone [18] were studied under the same experimental conditions.

MATERIALS AND METHODS

Brain serotonin. Female Charles River rats (180–200 g b.w.) were kept at constant room temperature (22 ± 1°) and relative humidity (60%) for all experiments.

The animals were injected intraperitoneally with various doses of trazodone and sacrificed 2 hr after the injection. Another group received 50 mg/kg i.p. of trazodone and were sacrificed at various times after the injection. In a third experiment the animals were injected i.p. with one of the following drugs: chlorimipramine (Cl-IMI), desipramine (DMI) and various doses of trazodone. Thirty min later they received 15 mg/kg i.p. of *d,l*-fenfluramine and were sacrificed 2 hr after the fenfluramine injection. Finally, the effects of CPP, OTPA and the *N*-oxide of trazodone were studied under the same experimental conditions utilized for trazodone. After animal sacrifice the brains were quickly removed and frozen for the bio-chemical assay. Serotonin and 5-hydroxyindoleacetic acid (5-HIAA) were estimated fluorimetrically according to the method of Giacalone and Valzelli [19].

Studies with platelets. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared and [^{14}C]5-HT uptake and release measured essentially as described previously [16, 17]. The uptake of [^{14}C]5-HT by rat platelets *in vitro* was stopped (by placing the test tubes in melting ice) after 2 min incubation at 37°. Previous experiments had shown that the saturation level for [^{14}C]5-HT in rat platelets is reached within 1–2 min [17]. [^{14}C]5-HT was used at a final concentration of $5{\cdot}5 \times 10^{-7}$ M, corresponding to 0·028 μCi/ml. Compounds to be tested were dissolved, just before use, in isotonic saline. Final concentrations are indicated throughout for *in vitro* experiments.

For *in vivo* experiments, rats were injected i.p. with various doses of test compounds. Blood was obtained from ether anesthetized animals 15 min after drug administration. Preliminary experiments had indicated the absence of any significant effect in blood collected 60 min after drug administration. The uptake of [^{14}C]5-HT by platelets was studied in PRP as described above.

RESULTS

Brain serotonin. As shown in Table 1, trazodone, even at a dose of 50 mg/kg, does not significantly affect the levels of 5-HT and 5-HIAA in the brain. Moreover, no significant changes of brain 5-HT and 5-HIAA were found at various times after the intraperitoneal injection of trazodone, 50 mg/kg (Table 2).

Table 3 shows the effect of trazodone on the depletion of brain serotonin induced by fenfluramine. It

Table 1. Effect of various doses of trazodone on the levels of 5-HT and 5-HIAA in the rat brain

Treatment (mg/kg, i.p.)		Brain levels (μg/g ± S.E.) 5-HT	5-HIAA
Saline		0·38 ± 0·02	0·32 ± 0·02
Trazodone	6·25	0·31 ± 0·02	0·35 ± 0·01
Trazodone	12·50	0·33 ± 0·02	0·31 ± 0·01
Trazodone	25·00	0·34 ± 0·01	0·27 ± 0·01
Trazodone	50·00	0·32 ± 0·01	0·29 ± 0·04

Each value represents the mean ± S.E. of six animals.
Brain indoles were estimated 2 hr after the drug injection.

Table 2. Brain levels of 5-HT and 5-HIAA at various times after injection of trazodone

Time after injection (min)	Brain levels (μg/g ± S.E.) 5-HT	5-HIAA
0	0·41 ± 0·01	0·30 ± 0·01
30	0·42 ± 0·02	0·33 ± 0·01
60	0·46 ± 0·02	0·34 ± 0·01
120	0·44 ± 0·01	0·30 ± 0·01

Trazodone was injected i.p. at a dose of 50 mg/kg.
Each value is the mean ± S.E. of six animals.

can be observed that trazodone even at relatively low doses (6·2 mg/kg), is significantly able to antagonize the depletion of brain 5-HT induced by fenfluramine. This effect is also shown by Cl-IMI but not by DMI, when used at the same dose. As shown in Table 4 CPP but not OTPA or trazodone *N*-oxide significantly antagonized the effect of fenfluramine on brain serotonin.

Studies with platelets. Figure 1 shows that both trazodone and CPP when added *in vitro* to PRP inhibited the uptake of [^{14}C]5-HT by rat platelets. The degree of inhibition was concentration-dependent. CPP was slightly more active than trazodone.

The other two possible metabolites of trazodone (OTPA and *N*-oxide) were completely ineffective (data not reported in the figure). For the sake of comparison, the effect of Cl-IMI in the same experimental condition is represented in Fig. 1: it was about 10 times more active than CPP.

Table 3. Effect of chloroimipramine (Cl-IMI), desipramine (DMI) and trazodone on the depletion of brain 5-HT induced by d,l-fenfluramine

Treatment	Dose (mg/kg, i.p.)	5-HT (μg/g ± S.E.
Saline		0·33 ± 0·22
d,l-fenfluramine	15	0·14 ± 0·01*
Cl-IMI	10	0·28 ± 0·01
DMI	10	0·28 ± 0·02
Trazodone	6·25	0·30 ± 0·02
Trazodone	12·50	0·33 ± 0·02
Trazodone	25·00	0·33 ± 0·01
Cl-IMI + *d,l*-fenfluramine	10 + 15	0·23 ± 0·02†
DMI + *d,l*-fenfluramine	10 + 15	0·18 ± 0·02
Trazodone + *d,l*-fenfluramine	6·25 + 15	0·23 ± 0·02†
Trazodone + *d,l*-fenfluramine	12·50 + 15	0·24 ± 0·01†
Trazodone + *d,l*-fenfluramine	25·00 + 15	0·25 ± 0·03†

Each figure is the mean ± S.E. of six animals. Drugs were injected 30 min before *d,l*-fenfluramine and 5-HT was estimated 2 hr after fenfluramine injection. Analysis of variance and the extension of Duncan's test have been used for the statistical evaluation of the data.
* $P < 0·01$ in respect to saline-treated animals.
† $P < 0·01$ in respect to fenfluramine-treated animals.

Table 4. Effect of *m*-chlorophenylpiperazine HCl (CPP), oxotriazolepyridin propionic acid (OTPA) and *N*-oxide of trazodone on the depletion of brain serotonin (5-HT) by *d,l*-fenfluramine

Treatment	Dose (mg/kg, i.p.)	5-HT (μg/g ± S.E.)
Saline		0·43 ± 0·03
d,l-fenfluramine	15	0·24 ± 0·003*
CPP	25	0·46 ± 0·02
OTPA	25	0·42 ± 0·02
Trazodone *N*-oxide	25	0·37 ± 0·02
CPP + *d,l*-fenfluramine	25 + 15	0·43 ± 0·02†
OTPA + *d,l*-fenfluramine	25 + 15	0·23 ± 0·01
Trazodone *N*-oxide + *d,l*-fenfluramine	25 + 15	0·24 ± 0·01

Each figure is the mean ± S.E. of six animals.
Drugs were injected 30 min before *d,l*-fenfluramine and 5-HT was estimated 2 hr after fenfluramine injection.
Analysis of variance and the extension of Duncan's test have been used for the statistical evaluation of the data.
* $P < 0·01$ with respect to saline-treated rats.
† $P < 0·01$ with respect to fenfluramine-treated animals.

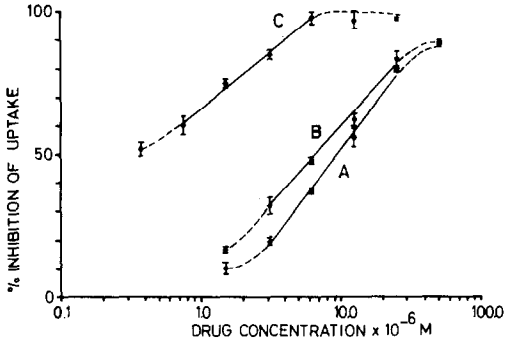

Fig. 1. Dose–response curves for the *in vitro* inhibitory activity of trazodone (curve A), *m*-chlorophenylipiperazine (curve B) and chlorimipramine (curve C) on [^{14}C]5-HT uptake by rat platelets. Each point represents the mean ± S.E. of at least four duplicate experiments. Drugs were preincubated with platelets for 15 min before an additional incubation period of 2 min with [^{14}C]5-HT.

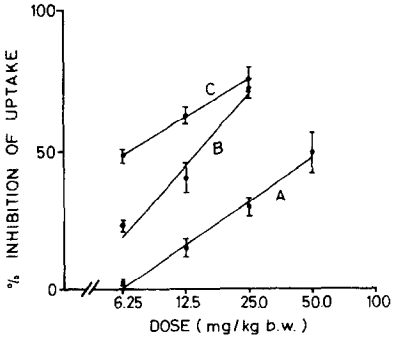

Fig. 2. Dose–response curves for the *in vivo* inhibitory activity of trazodone (curve A), *m*-chlorophenylpiperazine (curve B) and chlorimipramine (curve C) on subsequent *in vitro* [^{14}C]5-HT uptake by rat platelets. Each point represents the mean ± S.E. of at least four duplicate experiments. Drugs were given intraperitoneally to rats 15 min before blood collection. Platelets were subsequently incubated *in vitro* for 2 min with [^{14}C]5-HT.

Table 5. Dose-response curve for *in vitro* activity of trazodone and its proposed metabolites on [^{14}C]5-HT release from rat platelets

Drug	% Release (mean ± S.E.) at final concentration of		
	2.5×10^{-5} M	5×10^{-5} M	10^{-4} M
Trazodone	< 5 (8)*	5·5 ± 1·0 (6)	12·3 ± 0·1 (4)
m-chlorophenylpiperazine	5·7 ± 0·5 (4)	11·8 ± 0·7 (2)	15·8 ± 0·6 (4)
oxo-triazole-pyridinpropionic acid	< 5 (4)	< 5 (2)	
Trazodone *N*-oxide	< 5 (4)	< 5 (2)	
Chlorimipramine	< 5 (4)	14·3 ± 0·3 (4)	
(+)-Fenfluramine	30·6 ± 1·0 (14)	52·7 ± 3·0 (4)	

* Number in brackets is the number of experiments performed.

Figure 2 shows that trazodone, CPP and Cl-IMI given intraperitoneally to rats, inhibited the subsequent *in vitro* uptake of [^{14}C]5-HT by platelets. The inhibitory activity was dose-dependent and showed the same rate of potency as observed in the *in vitro* experiments.

Table 5 shows that neither trazodone, nor any of its proposed metabolites, induced an appreciable release of platelet-bound [^{14}C]5-HT after 2 hr of incubation at 37°. As previously reported [17], under similar experimental conditions, Cl-IMI was also ineffective, whereas *d*-fenfluramine induced significant release.

DISCUSSION

The present data demonstrate that trazodone, like Cl-IMI, does not affect the levels of 5-HT and 5-HIAA in the rat brain. At the same time, they show that trazodone is able to antagonize the decrease of brain 5-HT induced by fenfluramine. This effect is shared also by tricyclic antidepressant agents mainly the tertiary amines [13] and it has been interpreted as a consequence of the blockade exerted by these drugs on the membrane pump for transport into the brain serotonergic neurons of compounds able to release 5-HT [10, 12]. Assuming that fenfluramine utilizes this membrane pump, Cl-IMI would inhibit the entry of fenfluramine into the serotonergic neurons, therefore blocking the depletion effect of this drug on 5-HT stores.

Therefore, the present experiments indicate that trazodone and its congener *m*-chlorophenylpiperazine can share with tricyclic antidepressants the property of blocking the membrane uptake for serotonin in the brain. This possibility is supported by the observation that trazodone and *m*-chlorophenylpiperazine, similarly to tricyclic antidepressant agents [16], inhibit the uptake of 5-HT by platelets both *in vitro* and *in vivo*. It cannot be excluded however at the present time that the interaction between trazodone and fenfluramine is the result of a reduced availability of fenfluramine in the brain due to an alteration of its disposition and/or its metabolism.

The storage and the release mechanism for serotonin in the brain does not seem to be affected by either of the drugs, since no significant changes of 5-HT or 5-HIAA concentrations in the brain were observed following the administration of these compounds. Accordingly, *in vitro* studies show that neither trazodone nor *m*-chlorophenylpiperazine release significant amounts of 5-HT from platelets. It should be recalled that while *m*-chlorophenylpiperazine has never been detected after trazodone administration, two other compounds, oxatriazolepyridinpropionic acid [18] and trazodone *N*-oxide [1] identified as metabolites of trazodone are inactive as inhibitors of 5-HT uptake or as releasers of 5-HT on rat platelets. The potentiation of the central effects of 5-hydroxytryptophan, a precursor of serotonin, observed after the administration of trazodone [5, 6] may be related to the fact that trazodone inhibits the uptake of 5-HT.

In conclusion, it appears that trazodone and *m*-chlorophenylpiperazine inhibit the membrane uptake mechanism for serotonin. This finding may be of importance for interpreting the clinical antidepressant properties shown by trazodone [20, 21].

REFERENCES

1. L. Baiocchi, A. Chiara, A. Frigerio and P. Ridolfi, *Arzneimittel-Forsch.* **23**, 400 (1973).
2. G. Palazzo, *Curr. ther. Res.* **15**, 745 (1973).
3. B. Silvestrini, V. Cioli, S. Burberi and B. Catanese, *Int. J. Neuropharmac.* **7**, 587 (1968).
4. B. Silvestrini and R. Lisciani, *Curr. ther. Res.* **15**, 749 (1973).
5. J. R. Boissier, E. Portmann-Cristesco, P. Soubrié and J. Fichelle, in *Modern Problems of Pharmacopsychiatry*, Vol. 9, *Trazodone* (Eds. Th. A. Ban and B. Silvestrini), p. 18. Karger, Basel (1974).
6. V. G. Longo and A. Scotti de Carolis, in *Modern Problems of Pharmacopsychiatry*, Vol. 9, *Trazodone* (Eds. Th. A. Ban and B. Silvestrini), p. 4. Karger, Basel (1974).
7. S. Garattini, in *Modern Problems of Pharmacopsychiatry*, Vol. 9, *Trazodone* (Eds. Th. A. Ban and B. Silvestrini), p. 29. Karger, Basel (1974).
8. L. Angelucci and P. Bolle, in *Modern Problems of Pharmacopsychiatry*, Vol. 9, *Trazodone* (Eds. Th. A. Ban and B. Silvestrini), p. 65. Karger, Basel (1974).
9. K. Yamatsu, T. Kaneko, Y. Yamanishi, A. Kitahara and S. Ohtake, in *Modern Problems of Pharmacopsychiatry*, Vol. 9, *Trazodone* (Eds. Th. A. Ban and B. Silvestrini), p. 11. Karger, Basel (1974).
10. A. Carlsson, H. Corrodi, K. Fuxe and T. Hökfelt, *Eur. J. Pharmac.* **5**, 357 (1969).
11. S. B. Ross and A. L. Renyi, *Eur. J. Pharmac.* **7**, 270 (1969).
12. P. Lidbrink, G. Jonsson and K. Fuxe, *Neuropharmacology* **10**, 521 (1971).
13. D. Ghezzi, R. Samanin, S. Bernasconi, G. Tognoni, M. Gerna and S. Garattini, *Eur. J. Pharmac.* **24**, 205 (1973).
14. M. K. Paasonen, *J. Pharm. Pharmac.* **17**, 681 (1965).
15. A. Pletscher, *Br. J. Pharmac. Chemother.* **32**, 1 (1968).
16. W. Buczko, G. de Gaetano and S. Garattini, *J. Pharm. Pharmac.* **26**, 814 (1974).
17. W. Buczko, G. de Gaetano and S. Garattini, *Br. J. Pharmac.* **53**, 563 (1975).
18. T. Fujita, C. Yamato, T. Takahashi and S. Ohtake, in *Modern Problems of Pharmacopsychiatry*, Vol. 9, *Trazodone* (Eds. Th. A. Ban and B. Silvestrini), p. 56. Karger, Basel (1974).
19. E. Giacalone and L. Valzelli, *Pharmacology* **2**, 171 (1969).

20. A. Agnoli, M. Piccione, M. Casacchia and C. Fazio, in *Modern Problems of Pharmacopsychiatry*, Vol. 9, *Trazodone* (Eds. Th. A. Ban and B. Silvestrini), p. 190. Karger, Basel (1974).

21. G. B. Cassano, P. Castrogiovanni and L. Conti, in *Modern Problems of Pharmacopsychiatry*, Vol. 9, *Trazodone* (Eds. Th. A. Ban and B. Silvestrini), p. 199. Karger, Basel (1974).