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Effect of some anorectic agents on the uptake and release of 5-hydroxytryptamine by blood platelets of rats

Previously fenfluramine has been shown to differ from amphetamine at $2{\cdot}5 \times 10^{-5}$M in that it inhibits 5-hydroxytryptamine (5-HT) uptake and releases 5-HT from rat platelets (Buczko, de Gaetano & Garattini, 1975). Whether other anorectic agents share with fenfluramine its effects on rat platelet 5-HT has now been investigated.

Male Charles River rats (170–200 g) were used. Platelet-rich plasma (PRP) and platelet-poor plasma were prepared and ^{14}C-5-HT uptake and release measured according to Buczko, de Gaetano & Garattini (1974, 1975).

The drugs used were mazindol (5(*p*-chlorophenyl)-2-5-dihydro-3H-imidazo(2,1-α)-isoindol-5-ol) (Sandoz); SKF 39728-A (1-*N*-benzyl-β-methoxy-3-trifluoro-methylphenethylamine) and 4-chloroamphetamine HCl (Smith, Kline and French, Philadelphia, U.S.A.); (+)-fenfluramine HCl and S 992 [trifluoro-methylphenyl(benzoyloxy)-ethylamino-2-propane] (Servier Labs., Paris); phentermine (α-dimethylphenylethylamine) (Pennwalt, Rochester, USA); diethylpropion(2-diethylaminopropiophenone) (Richardson Merrell, Naples, Italy); (+)-amphetamine sulphate (Recordati, Milan, Italy).

Table 1 shows that, as found by Richter & Smith (1974) with human platelets, several anorectic drugs including phentermine, diethylpropion, SKF 39728-A and (+)-amphetamine are not capable of inhibiting 5-HT uptake and neither are they able to release 5-HT from rat platelets *in vitro*, as does fenfluramine.

S 992, a congener of fenfluramine, is inactive as an inhibitor of 5-HT uptake, while it is a weak releaser of 5-HT from platelets. Mazindol is comparable to fenfluramine in its capacity to inhibit 5-HT uptake but it too is a weak releaser of 5-HT. 4-Chloroamphetamine is more effective than fenfluramine on both parameters.

Table 2 shows a dose-response for mazindol and 4-chloroamphetamine on 5-HT uptake. Table 3 shows that both drugs strongly inhibit the initial rate of uptake; the

Table. 1. *Comparison of the effect of different anorectic drugs on ^{14}C-5-HT uptake (after 15 min) and release (after 2 h) from rat platelets.* Figures represent the mean ± s.e. of at least 4 different experiments.

Drug ($2{\cdot}5 \times 10^{-5}$M)	% Inhibition*	% Release**
(+)-Fenfluramine hydrochloride	52·0 ± 2·8	38·5 ± 1·3
(+)-Amphetamine sulphate	<5	<5
S 992	<5	10·5 ± 0·6
SKF 39728-A	<5	<5
Phentermine	<5	<5
Diethylpropion	<5	<5
Mazindol	50·1 ± 1·8	10·0 ± 0·7
4-Chloroamphetamine	70·1 ± 1·1	61·7 ± 1·2

* In respect to the uptake in controls.
** In respect to the 5-HT present in the control platelets at the end of the experiment.

platelets, however, continue to accumulate ^{14}C-5-HT during the incubation period. A similar pattern in the shape of the ^{14}C-5-HT uptake curves was obtained with fenfluramine (Buczko & others, 1975).

These results can be compared with data obtained *in vivo* on brain 5-HT. Phentermine, diethylpropion and mazindol do not affect brain 5-HT or 5-HIAA concentrations in rats (Samanin, unpublished); similarly negative results have been obtained for SKF 39728-A (Groppetti, Misher & others, 1972): S 992, which is ineffective in our *in vitro* studies on platelets, is effective in reducing brain 5-HT (Duhauft & Verdavainne, 1967; Garattini, Bizzi & others, 1974); this discrepancy, however, may be explained considering that this agent is rapidly metabolized *in vivo* to fenfluramine and norfenfluramine (Beckett, Shenoy & Brookes, 1972). Finally, 4-chloroamphetamine is active in lowering brain 5-HT and 5-HIAA concentrations (Pletscher, Bartholini & others, 1964; Fuller, Hines & Mills, 1965). However, in interpreting the long-lasting effect of this drug, the inhibition of 5-HT synthesis (Sanders-Bush & Sulser, 1970a, b) should also be considered. The capacity of 4-chloroamphetamine to release 5-HT from platelets had already been observed by Da Prada, Bartholini & Pletscher (1965) who used plasma-free rabbit platelets.

It appears, therefore, that the *in vivo* effects of anorectic drugs on brain 5-HT

Table 2. *Dose-response curves for the inhibitory activity of 4-chloroamphetamine and mazindol on ^{14}C-5-HT uptake by rat platelets.* Drugs were preincubated with PRP for 15 min before and for an additional incubation period of 15 min with ^{14}C-5-HT. Figures represent the mean and the ranges of at least two experiments.

Drug concentration ($\times 10^{-5}$M)	% Inhibition of ^{14}C-5-HT uptake	
	Mazindol	4-Chloroamphetamine
0·62	15·1 (14·8–15·5)	43·6 (41·6–46·7)
1·25	28·7 (28·5–28·9)	51·0 (45·6–56·5)
2·5	50·1 (48·1–55·2)	70·1 (68·9–73·2)

Table 3. *Kinetics of the uptake of ^{14}C-5-HT by rat blood platelets. Effect of chloroamphetamine* ($2{\cdot}5 \times 10^{-5}$M) *and mazindol* ($2{\cdot}5 \times 10^{-5}$M). The figures represent the mean of two experiments.

Time after addition of 0·05 μg ^{14}C-5-HT	% Uptake of ^{14}C-5-HT		
	Control	4-Chloroamphetamine	Mazindol
10 s	65	0	7·0
20 s	70	2·2	7·8
30 s	90	6·5	12·3
1 min	95	5·6	11·2
2 min	95	6·1	15·2
3 min	95	12·7	18·7
5 min	95	15·3	24·2
10 min	95	14·4	33·4
15 min	95	28·9	48·3

Subsamples of the same PRP were preincubated with either drugs for 15 min before the addition of ^{14}C-5-HT; at each time interval indicated a subsample was rapidly cooled by placing it in melting ice; at the end of 15 min all the specimens were centrifuged at 3000 *g* for 15 min in a cold room. The amount of radioactivity remaining in the supernatant plasma was measured and uptake was calculated according to Buczko & others (1974).

concentrations are correlated much better with the capacity of these drugs to release 5-HT from platelets *in vitro* than with their *in vitro* inhibitory activity on platelet 5-HT uptake.

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The role of the raphé and extrapyramidal nuclei in the stereotyped and circling responses to quipazine

The induction of stereotyped behaviour patterns in laboratory animals is widely used as an index of dopaminergic stimulation and, therefore, as an indicator of antiparkinson potential (Costall, Naylor & Wright, 1972; Fog, 1972; Costall, Naylor & Pinder, 1974). However, the ability of dopaminergic agonists to cause stereotypy has also recently been associated with serotoninergic (5-HT) mechanisms (Costall & Naylor, 1974a) and this raises questions as to the possible relation between dopamine and 5-HT in parkinsonism. Reports that quipazine causes stereotyped behaviour patterns but acts mainly upon 5-HT mechanisms (Rodriguez & Pardo, 1971; Medon, Leeling & Phillips, 1973; Grabowska, Antkiewicz & Michaluk, 1974) are, therefore, of great interest and stimulated the present studies to investigate its action using brain lesion and intrastriatal injection techniques.

Male Sprague-Dawley rats, 250—300 g, were used. In these animals quipazine (2-(1-piperazinyl) quinoline maleate) (Miles Laboratories) did not induce behavioural effects analogous to the stereotypy induced by known dopaminergic agonists, for example, apomorphine and (+)-amphetamine. The intensity of stereotyped behaviour induced by the latter agents generally increases with increasing dosage such that the weak intensity components of sniffing and repetitive limb movements are apparent at lower doses, and the intense components of gnawing, biting and licking at higher doses