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Effect of two non tricyclic antidepressant drugs on [^{14}C]5-hydroxytryptamine uptake by rat platelets

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The uptake of ^{14}C-5-HT by rat blood platelets was examined *in vitro* in experimental conditions which allowed measurement of the initial velocity and excluded other passive processes across the cell membrane. In these conditions, the effect of two non tricyclic antidepressant drugs (Lilly 110140 and trazodone) was investigated. Lilly 110140 was as active as chlorimipramine and several times more active than imipramine as an inhibitor of ^{14}C-5-HT uptake. Like chlorimipramine, Lilly 110140 appeared to be either a non-competitive or an uncompetitive inhibitor, according to the concentration of drug used. Trazodone also inhibited ^{14}C-5-HT uptake by platelets but to a lesser extent than chlorimipramine, imipramine or Lilly 110140. *m*-Chlorophenylpiperazine, a possible metabolite of trazodone, was about 3 times more potent an inhibitor than the parent molecule. Both compounds acted non-competitively. Compared with published data on the effect of Lilly 110140 and trazodone on brain 5-HT, the present results support the suggestion that rat platelets are a useful pharmacological model of serotoninergic nerve endings.

Blood platelets have been considered to be a useful model for serotoninergic nerve endings and have been used in the study of the inhibitory effect on 5-hydroxytryptamine (5-HT) uptake of a number of tricyclic antidepressant drugs (Pletscher, 1968; Buczko, de Gaetano & Garattini, 1974; Tuomisto, 1974).

Recently, two new non-tricyclic compounds have been reported to interfere with 5-HT uptake in rat brain: Lilly 110140, 3-(*p*-trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine (Wong, Bymaster & others, 1975) and trazodone, 2-{3-[4(*m*-chlorophenyl)-1-piperazinyl] propyl}-5-triazolo [4,3-a]pyridin-3-(2H)one (Garattini, de Gaetano & others, 1976).

We have investigated the *in vitro* effect of both drugs on the kinetics of ^{14}C-5-HT uptake by rat platelets to better characterize their mechanism of action. A potential metabolite of trazodone, *m*-chlorophenylpiperazine (CPP) was also investigated.

MATERIALS AND METHODS

Blood obtained from male Sprague Dawley (Charles River) rats, 250–300 g, by intracardiac puncture, after slight ether-anaesthesia, was collected in 10 ml disposable plastic syringes containing 1 ml 0·126 M trisodium citrate. Platelet rich-plasma (PRP) was prepared as previously described (Buckzo, de Gaetano & Garattini, 1975). To fulfil the requirements of the initial velocity measurements (Tuomisto, 1974) all experiments were performed by limiting the incubation period of the platelets with ^{14}C-5-HT to 30 s. This time corresponded approximately to half of the period in which uptake appeared to be linear at all the substrate concentrations used.

Measurement of ^{14}C-5-HT uptake

Aliquots of 1·0 ml PRP were pipetted into 5 ml plastic tubes. Samples were preincubated at 37° for 10 min (the average time required to reach 37° for similar samples of PRP (Praga & Pogliani, 1973). After preincubation, 0·05 ml of twice distilled water or solution of test compound was added to PRP and incubation was continued for further 15 min when ^{14}C-5-HT (at different concentrations) was added and the incubation was continued for further 30 s. The incubation was stopped by chilling the tubes rapidly in melting ice; 0·2 ml aliquots of the samples were then transferred to counting vials containing 10 ml of a dioxane-naphthalene scintillation mixture.

The samples were then centrifuged at 4000 *g* for 15 min at 4°, and 0·2 ml aliquots of platelet-free supernatant were transferred to counting vials.

Radioactivity (d min^{-1}) was measured in a Packard Tri Carb Liquid Scintillation spectrometer (Model 3002) for 1 min. Uptake was expressed as d min^{-1}/10^8 platelets of incubation with ^{14}C-5-HT.

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Kinetics of ^{14}C-5-HT uptake and inhibition

For each concentration of the test compounds, concentrations of ^{14}C-5-HT from 0·25 to 2·0 μM were used.

Double reciprocal plots of ^{14}C-5-HT uptake by platelets were calculated by the method of least squares. Km (which represents the concentration of ^{14}C-5-HT giving half maximal uptake) and Vmax (which is the maximal entry rate) were first determined by Lineweaver and Burk plots. Both values were controlled by Woolf plots for enzyme kinetics. This analysis fits the Michaelis-Menten equation using the Woolf linear transformation $S/V = (Km + S)/Vmax$ and allows a suitable weighting of the experimental data. For the experiments in which Lilly 110140 was used, estimates of Km and Vmax values were also obtained by the method of bilinear regression described by Wilkinson (1961).

Both Lineweaver-Burk and Woolf plots were drawn by appropriate computer programs using a Hewlett Packard 9810A computer connected to a Hewlett Packard Plotter 9862 A.

IC50 values (i.e. the concentration of inhibitor producing 50% inhibition of ^{14}C-5-HT uptake) were extrapolated from log dose-response curves based on experiments in which platelets were incubated with varying concentrations of the inhibitor and a constant concentration of ^{14}C-5-HT (0·5 μM).

Ki values (i.e. the dissociation constant of the enzyme (platelet)-inhibitor complex) were calculated by the computer on the basis of the difference in the Vmax value (Cheng & Prusoff, 1973) which were obtained by Woolf plots.

Drugs were dissolved in twice distilled water. One series of experiments was performed by adding simultaneously both Lilly 110140 and ^{14}C-5-HT to PRP and stopping the uptake after 30 s by the addition of 1·5% formaldehyde (Costa & Murphy, 1975).

RESULTS

Lilly 110140, trazodone and its metabolite CPP all inhibited ^{14}C-5-HT uptake by rat platelets.

As shown in Table 1, the IC50 for Lilly 110140 was 0·28 μM, a value similar to that found for chlorimipramine, and more than four times less than that found for imipramine, which was our reference drug.

In contrast, trazodone had an IC50 of 8·5 μM which was about 6 times greater than that of imipramine. CPP appeared to be about 3 times more active than its parent molecule, its inhibitory potency being approximately half that of imipramine.

Table 1. *Inhibitory effect of different anti-depressant drugs on the uptake of ^{14}C-5-HT by rat platelets.* Means and 95% confidence limits of 3 experiments.

Compound	IC50 (μM)	Potency relative to imipramine = 100	Ki (μM)
Imipramine	1·25 (1·08–1·42)	100	1·78 (1·57–1·99)
Chlorimipramine	0·16 (0·07–0·25)	781	0·15 (0·10–0·20)
Lilly	0·28 (0·19–0·37)	446	0·29 (0·12–0·46)
Trazodone	8·50 (7·30–9·70)	15	8·50 (7·00–9·00)
CPP	3·00 (2·35–3·64)	42	2·80 (1·34–4·26)

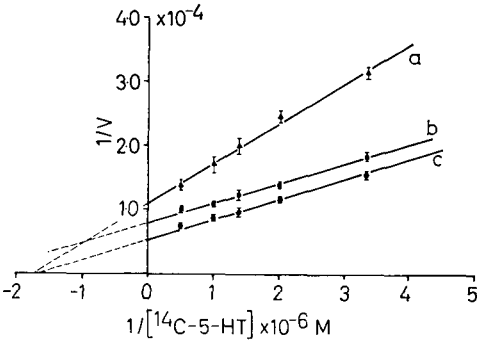

FIG. 1. Kinetic analysis of ^{14}C-5-HT uptake inhibition by Lilly 110140. Reciprocals of ^{14}C-5-HT concentrations were plotted *vs* its accumulation into platelets in the absence (c) and presence of Lilly 110140 b—1×10^{-7} M, a—5×10^{-7} M. Amine uptake velocity (V) is expressed as d $min^{-1}/10^8$ platelets. The experiment was repeated four times. S.e.m. values are shown by vertical bars.

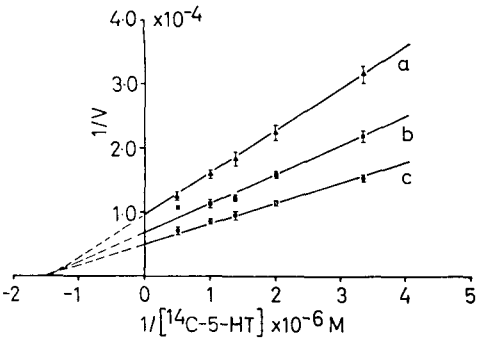

FIG. 2. Kinetic analysis of ^{14}C-5-HT uptake inhibition by trazodone. Reciprocals of ^{14}C-5-HT concentrations were plotted *vs* its accumulation into platelets in the absence (c) and presence of trazodone b—5×10^{-6} M, a—1×10^{-5} M. Amine uptake velocity (V) is expressed as d $min^{-1}/10^8$ platelets. The experiment was repeated four times. S.e.m. values are shown by vertical bars.

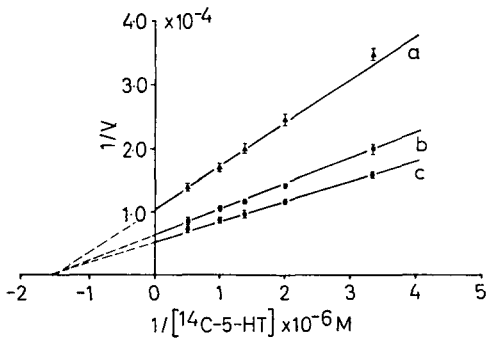

FIG. 3. Kinetic analysis of ^{14}C-5-HT uptake inhibition by *m*-chlorophenylpiperazine (CPP). Reciprocals of ^{14}C-5-HT concentrations were plotted *vs* its accumulation into platelets in the absence (c) and presence of *m*-chlorophenylpiperazine b—1×10^{-6} M, a—5×10^{-6} M. Amine uptake velocity (V) is expressed as d min^{-1}/10^8 platelets. The experiment was repeated four times. S.e.m. values are shown by vertical bars.

Figs 1–3 show the type of inhibition exerted by Lilly 110140, trazodone and CPP respectively. As can be seen from Lineweaver-Burk plots, they were all apparently non-competitive inhibitors of ^{14}C-5-HT uptake by rat platelets. The type of inhibition exerted by the different compounds was also found to be apparently non-competitive by both Woolf and Wilkinson analysis of the data (Table 2).

At the lower concentration (0·1 μM), Lilly 110140 appeared to act un-competitively (Fig. 1 and Table 2). Inhibition by both imipramine and chlorimipramine was apparently non-competitive; however, chlorimipramine, at 0·05 μM appeared to be an un-competitive inhibitor (Wielosz, Salmona & others, 1976).

Table 2. *Apparent Km and Vmax values for ^{14}C-5-HT uptake by platelets.* Effect of different anti-depressant drugs. Means ± s.e.m. of 4 experiments.

		Woolf analysis		Wilkinson analysis		
Compounds	Final concn (μM)	Km (μM)	Vmax d min^{-1} 10^8 platelets	Km (μM)	Vmax d min^{-1} 10^8 platelets	Type of inhib.
Control	—	0·615 ± 0·093	46 297 ± 4665	0·617 ± 0·134	47 108 ± 4925	—
Lilly 110140	0·1	0·277 ± 0·054	25 082 ± 1840	0·283 ± 0·058	25 437 ± 1683	Un-comp.
Lilly 110140	0·5	0·612 ± 0·125	22 351 ± 3040	0·650 ± 0·175	23 330 ± 3202	Non-comp.
Trazodone	5	0·728 ± 0·151	37 632 ± 5671			Non-comp.
Trazodone	10	0·821 ± 0·147	28 134 ± 3862			Non-comp.
CPP	1	0·612 ± 0·042	37 040 ± 1701			Non-comp.
CPP	5	0·847 ± 0·110	26 438 ± 2675			Non-comp.

As reported in Table 1, the Ki values were similar to the IC50 values, which confirms the non-competitive nature of inhibition exerted by the three compounds.

Table 3 reports the results of four experiments made without preincubation of platelets with Lilly 110140 before the addition of ^{14}C-5-HT. In these experiments, the uptake of the amine was stopped by

Table 3. *Apparent Km and Vmax values for ^{14}C-5-HT uptake by platelets.* Effect of Lilly 110140. Means ± s.e.m. of four experiments.

Compound	Km (μM)	Vmax d min^{-1} 10^8 platelets	Type of inhibition
Control	1·19 ± 0·10	50 394 ± 3191	—
Lilly 110140 (10^{-7}M)	0·77 ± 0·10	34 732 ± 2649	Un-competitive
Lilly 110140 (5×10^{-7}M)	1·41 ± 0·15	29 222 ± 2434	Non-competitive

adding 0·2 ml formaldehyde (1·5%, final concentration) as recently described by Costa & Murphy (1975). This technique allows a more rapid and complete arrest of the active uptake of ^{14}C-5-HT by platelets than that obtained by chilling the tubes. In these experimental conditions, Lilly 110140 (at the two concentrations used) acted as either a non-competitive or an uncompetitive inhibitor of ^{14}C-5-HT uptake.

The apparent Km values (obtained by Woolf plot) were slightly higher than those obtained in previous experiments.

DISCUSSION

Recent work has shown that Lilly 110140 is a potent inhibitor of uptake of 5-HT into the synaptosomes of rat brain (Wong & others, 1975). The present study demonstrates that Lilly 110140 is also a potent inhibitor of uptake of ^{14}C-5-HT into rat platelets. Wong & others (1975) observed that Lilly 110140 was as effective as chlorimipramine and 8 times more potent than imipramine in inhibiting the uptake of 5-HT into brain synaptosomes. Under their experimental conditions, IC50 values for Lilly 110140, chlorimipramine and imipramine were 0·06, 0·09 and 0·5 μM, respectively.

Although the IC50 values we obtained were slightly higher, they clearly show that on rat platelets Lilly 110140 was as active as chlorimipramine and several times more active than imipramine.

Our data are in agreement with the IC50 values recently reported by Drummond & Gordon (1975) working with rat platelets (0·5, 0·2 and 0·7 μM for Lilly 110140, chlorimipramine and imipramine,

respectively). Wong & others (1975) also reported that Lilly 110140 competitively inhibited 5-HT uptake into rat synaptosomes in experiments designed according to either the method of Dixon (1953) or to the Lineweaver-Burk method. However, from their data, the competitive nature of inhibition of 5-HT uptake by Lilly 110140 may be questioned. They reported a Ki value (0·055 μM) for Lilly 110140 that is identical to the IC50 value (0·06 μM) a finding typical of non-competitive or un-competitive types of inhibition (Cheng & Prusoff, 1973). Our results on platelets confirm that Lilly 110140 has very similar Ki and IC50 values (Table 1).

As far as trazodone and its metabolite CPP are concerned, this study confirms and extends our previous observation (Garattini & others, 1976) that both compounds inhibit 5-HT uptake by rat platelets to a lesser extent than imipramine and chlorimipramine. The relatively weaker effect of trazodone, compared with its metabolite CPP, has also been confirmed. In addition, the present study indicates that the inhibitory effect of both compounds is apparently non-competitive. To the best of our knowledge, no data have been published concerning the effect of either compound on 5-HT uptake into rat brain synaptosomes, although there are indirect *in vivo* data supporting an inhibition of 5-HT uptake in the brain (Garattini & others, 1976).

A very recent study by Horng & Wong (1976) has reported that Lilly 110140 inhibits 5-HT uptake in platelets of rats and man. Although the experimental conditions we used differed in many respects from those of Horng & Wong (1976), the results obtained were essentially the same. Indeed, those authors found that in inhibiting ^{14}C-5-HT uptake into rat platelets, chlorimipramine was the most potent, followed by Lilly 110140 and imipramine with IC50 values of 0·04, 0·10 and 0·16 μM, respectively. The type of inhibition was not determined.

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