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The effect of drugs upon the uptake of 5-hydroxytryptamine and metaraminol by human platelets

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The abilities of some tricyclic and bicyclic antidepressive drugs and an α-receptor blocking agent, phenoxybenzamine, to inhibit the uptake of 5-hydroxytryptamine (5-HT) and (−)-metaraminol into human platelets have been compared *in vitro*. All the drugs inhibited the uptake both of 5-HT and of metaraminol into platelets. But there were differences in their abilities to inhibit the uptake of these two monoamines. The desmethylated antidepressive drugs were more potent inhibitors of metaraminol uptake than were their tertiary analogues, whereas imipramine, a tertiary amine, was by far the best inhibitor of 5-HT uptake. The order of the activities of the antidepressive drugs in inhibiting the uptake of 5-HT and metaraminol into platelets paralleled their potencies in blocking the uptake of 5-HT, and noradrenaline or metaraminol into nerve endings. It is suggested that the uptake of 5-HT and metaraminol into platelets is a useful model for the neuronal uptake of 5-HT and noradrenaline, respectively.

Uptake of monoamines into blood platelets can be inhibited by various drugs among which the most potent are the tricyclic antidepressants (Paasonen, 1965; 1968). Of these the tertiary amines are the more potent inhibitors of 5-hydroxytryptamine (5-HT) uptake (Yates, Todrick & Tait, 1964; Ahtee, Tuomisto & others, 1968; Todrick & Tait, 1969). The secondary amines are more potent as inhibitors of the uptake of noradrenaline into central and peripheral neurons (Callingham, 1966; Iversen, 1967; Carlsson, Corrodi & others, 1969b). Carlsson, Corrodi & others (1969a) recently demonstrated the tertiary amines to be more potent in blocking the uptake of 5-HT into central neurons than corresponding secondary amines. In the present work the effects of several antidepressive drugs on the uptake of 5-HT and (−)-metaraminol into human blood platelets has been examined. Metaraminol was used as a model for sympathomimetic amines since it has a high affinity for the storage sites of noradrenaline (Andén, 1964; Shore, Busfield & Alpers, 1964; Carlsson & Waldeck 1965).

MATERIALS AND METHODS

Buffy coats from 400 ml human citrated blood were diluted with one volume of modified calcium-free Tyrode solution (g/litre: disodium edetate 0·8, NaCl 7·6, KCl 0·42, $NaH_2PO_4x2\ H_2O$ 0·14, $NaHCO_3$ 2·1, glucose 2·0 and sucrose 4·5) and platelets were separated by centrifuging at about 130 *g* for 20 min at 20°. The final dilution of the platelet suspension contained $8{\cdot}86 \pm 0{\cdot}41 \times 10^8$ platelets/ml (means ± s.e. from 53 experiments) and about 1/10 of the medium was original plasma.

If not otherwise stated, duplicate samples (2 ml) of the platelet suspension were incubated for 15 min with gentle shaking at 37° with or without 10^{-5}M of 5-hydroxytryptamine creatinine sulphate (Fluka AG) or 3×10^{-6}M of (−)-metaraminol bitartrate (Merck Sharp & Dohme), in oxygen containing 4% carbon dioxide. At these extracellular concentrations of the monoamines, the platelets took up sufficient of the monoamines for them to be accurately estimated spectrophotofluorometrically in the samples taken.

The hydrochlorides of imipramine and desipramine (Geigy A.G.), protriptyline (Merck Sharp & Dohme), and 1-(3-methylaminopropyl)-1-phenyl-3,3-dimethylphthalane (Lu 3-010), and its corresponding tertiary amine (Lu 3-009) (H. Lundbeck & Co. A/S), and of phenoxybenzamine (Smith Kline & French Labs.) were added to the suspension 10 min before 5-HT or metaraminol. All drugs were dissolved in saline and added in a volume of 0·2 ml. Only polypropylene vessels and pipettes were used to handle platelets.

After incubation, the platelets were cooled immediately to below 5° and centrifuged for 20 min at 2500 *g*. The supernatant was decanted and traces remaining in the incubation tubes were removed with a filter paper. The platelet pellet was then lysed in 1·5 ml of distilled water in a vortex mixer.

The proteins were precipitated with 10% $ZnSO_4$ (0·2 ml) and N NaOH (0·1 ml). The mixture was shaken well and centrifuged for 5 min at 700 *g*. After the addition of 12N HCl (0·3 ml), the 5-HT was measured from 1 ml of the supernatant spectrophotofluorometrically. Metaraminol was measured by the *o*-phthaldialdehyde procedure of Shore & Alpers (1964). None of the drugs studied interfered with the fluorescence of 5-HT or metaraminol.

The means and standard errors (s.e.) were calculated and the statistical significance of the differences was determined by Student's *t*-test. The concentrations of the drugs which caused 50% inhibition of monoamine uptake (IC50) were determined by using straight lines obtained by the method of least squares.

RESULTS

Uptake of metaraminol into platelets

Table 1 shows the uptake of metaraminol into, and release of 5-HT from, human blood platelets as a function of extracellular metaraminol concentration. The

Table 1. *Uptake of (−)-metaraminol (MA) by and release of 5-HT from human blood platelets incubated with various concentrations of MA.* Duplicate 2-ml samples of platelet suspensions were incubated for 1 h at 37°. Means ± s.e. from 4 experiments.

Added MA (M)	MA taken up nmol/10^9 platelets	Concentration gradient*	5-HT release nmol/10^9 platelets**
10^{-6}	0·4 ± 0·04	40	0
3×10^{-6}	1·2 ± 0·2	39	0
10^{-5}	2·8 ± 0·4	28	0
3×10^{-5}	4·5 ± 0·7	15	0·24 ± 0·07
10^{-4}	8·8 ± 1·5	9	0·63 ± 0·17

* MA in 10^{11} platelets (ml packed platelets)/MA in ml of incubation medium.
** Original 5-HT content 3·94 ± 0·72 nmol/10^9 platelets.

platelets took up metaraminol against a concentration gradient which was inversely proportional to its concentration in the external medium. No release of platelet 5-HT was observed below 3×10^{-5}M metaraminol which released 6% of platelet 5-HT in 1 h.

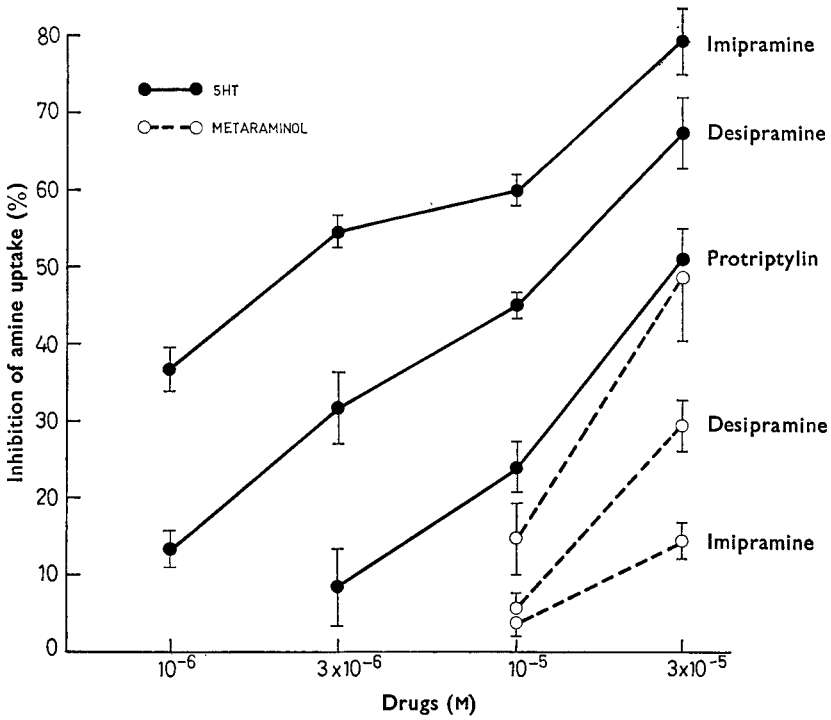

FIG. 1. Inhibition of 5-hydroxytryptamine (5-HT) and (−)-metaraminol (MA) uptake into human blood platelets by tricyclic antidepressants. Means ± s.e. from 5 to 13 experiments.

Inhibition of monoamine uptake into platelets by drugs

Fig. 1 shows the effects of imipramine, desipramine and protriptyline on the uptake of 5-HT and metaraminol into platelets. At 10^{-6}M, both imipramine and desipramine significantly inhibited 5-HT uptake ($P < 0{\cdot}001$) and the inhibition increased with increasing concentrations of the compounds. Imipramine, a tertiary amine, was a more potent inhibitor than the corresponding secondary amine, desipramine ($P < 0{\cdot}001$ at 10^{-6}, 3×10^{-6} and 10^{-5}M). Protriptyline inhibited 5-HT uptake even less than desipramine. The order of potency of these drugs in blocking the metaraminol uptake was protriptyline > desipramine > imipramine which is opposite to that of their inhibition of 5-HT uptake. Phenoxybenzamine inhibited the uptake of metaraminol slightly more than it did that of 5-HT (Table 2).

Table 3 shows the concentrations of different drugs that caused 50% inhibition (IC50) of 5-HT and metaraminol uptake. It also gives the ratios of IC50 of metaraminol uptake to that of 5-HT uptake. Imipramine was by far the best inhibitor of 5-HT uptake but was a relatively weak inhibitor of metaraminol uptake. To inhibit the uptake of metaraminol to the same extent as that of 5-HT, a concentration of imipramine over 300 times higher was needed. Also desipramine more effectively inhibited the uptake into platelets of 5-HT than of metaraminol.

Table 2. *Inhibition of 5-HT and MA uptake into human blood platelets by phenoxybenzamine.* Means ± s.e., number of experiments in brackets.

Phenoxybenzamine (M)	Inhibition of amine uptake (%) 5-HT uptake	MA uptake
10^{-6}	8·8 ± 2·8 (7)	0·4 ± 6·7 (3)
3×10^{-6}	16·5 ± 4·8 (7)	27·3 ± 8·5 (5)
10^{-5}	50·3 ± 4·8 (7)	58·9 ± 3·8 (7)
3×10^{-5}	62·6 ± 3·6 (7)	74·1 ± 5·8 (5)

Table 3. *Inhibition of 5-HT and MA uptake into human blood platelets by drugs.* Concentrations causing 50% inhibition (IC50) of monoamine uptake were determined from means of three to ten experiments using at least four different concentrations of drugs. Last column gives the ratios of IC50 for MA uptake to that of 5-HT uptake.

Drug	IC50 (M) 5-HT uptake	MA uptake	IC50 MA / IC50 5-HT
Imipramine	$2{\cdot}86 \times 10^{-6}$	$9{\cdot}63 \times 10^{-4}$	337
Desipramine	$1{\cdot}09 \times 10^{-5}$	$7{\cdot}80 \times 10^{-5}$	7·2
Protriptyline	$4{\cdot}64 \times 10^{-5}$	$3{\cdot}20 \times 10^{-5}$	0·7
Lu 3-009	$5{\cdot}77 \times 10^{-5}$	$4{\cdot}08 \times 10^{-5}$	0·7
Lu 3-010	$2{\cdot}12 \times 10^{-5}$	$2{\cdot}39 \times 10^{-5}$	1·2
Phenoxybenzamine	$1{\cdot}35 \times 10^{-5}$	$8{\cdot}16 \times 10^{-6}$	0·6

The secondary amine protriptyline was about thirty times more potent than imipramine and twice as potent as desipramine in inhibiting metaraminol uptake. It inhibited metaraminol uptake in concentrations approximately similar to those needed to inhibit the 5-HT uptake.

The bicyclic compound with a secondary amine group, Lu 3-010, was even more potent inhibitor of metaraminol uptake than was protriptyline. It inhibited both metaraminol and 5-HT uptake more than its tertiary amine analogue Lu 3-009. The most potent inhibitor of metaraminol uptake in these experiments was phenoxybenzamine.

DISCUSSION

Our results show that human blood platelets are able to accumulate (−)-metaraminol against a concentration gradient with a saturable process. The concentration gradient is higher than that which has been shown for (±)-noradrenaline (Abrams & Solomon, 1969) or for (±)-adrenaline (Born & Smith, 1970). As the release of 5-HT did not occur until after the platelets had taken up about as many mol of metaraminol as they contain 5-HT, there seems to be, in human platelets, a space where metaraminol is accumulated before it starts replacing 5-HT. Rabbit platelets contain about 15–20 times as much 5-HT as human platelets and from these metaraminol releases 5-HT stoichiometrically (Ahtee & Saarnivaara, unpublished results). This discrepancy could be explained by the fact that human platelets accumulate *in vitro* many times their original content of 5-HT whereas rabbit platelets are more saturated. Both in human and rabbit platelets most of the accumulated metaraminol can be released by thrombin and therefore it is not localized in the cytoplasm but in a

bound form most probably in the storage granules (Ahtee & Mills, unpublished results).

All the drugs studied inhibited both the uptake of 5-HT and metaraminol into platelets. There were, however, differences in their abilities to inhibit the uptake of these two monoamines. The most striking was the 300-fold difference in concentrations of imipramine needed to inhibit the uptake of 5-HT and metaraminol. These results agree with those of Carlsson, Corrodi & others (1969 a,b) and Carlsson, Fuxe & others (1969) who used a displacement technique to show that the abilities of several tricyclic and bicyclic compounds to block the uptake of monoamines into central and peripheral noradrenaline neurons were dissociated from their abilities to block uptake into central 5-HT neurons.

As has been previously shown (Yates & others, 1964; Ahtee & others, 1968; Todrick & Tait, 1969) the tricyclic antidepressant with a tertiary amine structure, imipramine, was a more potent inhibitor of 5-HT uptake than its *N*-desmethyl derivative, desipramine or the secondary amine, protriptyline. Of the two bicyclic compounds studied, however, the one with a secondary amine structure, Lu 3-010, inhibited 5-HT uptake in lower doses than the corresponding tertiary amine, Lu 3-009. The potency of the bicyclic compounds in inhibiting 5-HT uptake was about the same as that of desipramine and protriptyline. The uptake of metaraminol into platelets was more strongly inhibited by the secondary amines, Lu 3-010, protriptyline and desipramine, than their corresponding tertiary derivatives, Lu 3-009 and imipramine. A similar superiority of the desmethyl series of antidepressive drugs in inhibiting the uptake of noradrenaline or metaraminol into peripheral ($uptake_1$) or central neurons has been demonstrated previously (Callingham, 1966; Waldeck, 1968; Carlsson, Corrodi & others, 1969b; Carlsson & others, 1969).

In relatively high concentrations *in vitro* the tricyclic antidepressants and related drugs liberate 5-HT and other cellular components from the platelets causing membrane damage. They also haemolyse red cells. The antidepressive drugs containing a secondary amine are more potent 5-HT releasers than are the tertiary amines, although the tertiary amines cause haemolysis in lower concentrations than the secondary amines (Paasonen, 1964; Ahtee, 1966; Solatunturi, 1968; Ahtee & Paasonen, 1968a, b). The effects of these drugs on the membrane of two different cells thus differ and moreover the 5-HT uptake-inhibiting and 5-HT-releasing effects in the platelets are not parallel. Furthermore, we have now demonstrated that these drugs inhibit the uptake of the two monoamines, 5-HT and metaraminol in different ways. The monoamines are most probably transported through the cell membrane by means of carriers or through uptake sites. Several drugs, e.g. the tricyclic antidepressants, affect these transport mechanisms. Our results indicate that these drugs inhibit the uptake of 5-HT and metaraminol into the platelets in the same way as they inhibit the uptake of 5-HT and noradrenaline into neurons. It is likely that the same carriers or uptake sites (or both) are responsible for the pumping of both monoamines into platelets with the various drugs obstructing the pump in different ways, one drug leaving more room for one monoamine to pass through than for another.

Phenoxybenzamine, an α-blocking drug, was the most potent inhibitor of metaraminol uptake in our experiments. It inhibited the uptake of metaraminol slightly more than it did that of 5-HT. Similar results are reported by Bygdeman & Johnsen (1969) who found that higher concentrations of α- and β-blockers were needed to

inhibit the uptake of 5-HT than that of noradrenaline into platelets. Phenoxybenzamine is a potent inhibitor of the uptake of monoamines into granules (Euler & Lishajko, 1968). This effect might be partially responsible for its inhibitory activity in our experiments which did not distinguish between the membrane and granular part of the monoamine uptake.

Recently, Iversen & Langer (1969) showed that phenoxybenzamine usually prevents the uptake of noradrenaline competitively. However, in circumstances that resembled ours, phenoxybenzamine behaved as a non-competitive inhibitor of noradrenaline uptake into vas deferens. Iversen & Langer showed that in addition to preventing the uptake of noradrenaline into sympathetic nerve endings ($uptake_1$), phenoxybenzamine inhibited the uptake of noradrenaline into the extraneuronal cells ($uptake_2$). In the perfused rat heart these two membrane mechanisms ($uptake_1$ and $uptake_2$) are inhibited in different ways by various drugs, metaraminol possessing the highest affinity for $uptake_1$ (Iversen, 1967). Lahovaara, Neuvonen & Paasonen (1970) could demonstrate neither the inhibitory specifity of $uptake_1$ nor that of $uptake_2$ in the uptake of noradrenaline into human platelets. It is plausible, however, that the uptake of monoamines into platelets, which occurs both through the membrane and into the granules, and operates against a concentration gradient of hundreds (5-HT) or tens (metaraminol), resembles more the true neuronal uptake than the extraneuronal accumulation of monoamines.

We suggest that the effects of drugs on the uptake of different monoamines into platelets could be used as a model to study drug effects on the uptake of different monoamines into neurons. The platelet is suitable serving as a model for 5-HT uptake but more knowledge can be gained from experiments studying also the uptake of other monoamines into platelets. We have recently found a dissociation in the effects of several analgesic drugs on 5-HT and metaraminol uptake into platelets (Ahtee & Saarnivaara, 1970).

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