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Effect of imipramine and some analogues on the uptake of 5-hydroxytryptamine by human blood platelets *in vitro*

CELIA M. YATES,* A. TODRICK AND A. C. TAIT

The inhibitory action of imipramine and some analogues on the uptake of 5-hydroxytryptamine by human blood platelets *in vitro* was investigated. In concentrations comparable with those reached in plasma during therapy, imipramine and the isosteric compound, amitriptyline, were more effective than their metabolites. Chlorpromazine was less effective than the latter and orphenadrine ineffective at the concentrations tested.

BIOCHEMICAL studies on the mode of action of drugs used in the treatment of depression have largely been related to the effects on catecholamine and 5-hydroxytryptamine (5-HT) metabolism. The substituted hydrazine class of monoamine oxidase inhibitors, for example, causes a rise in the brain amine levels of experimental animals (Spector, Prockop, Shore & Brodie, 1958).

In man, the amine which can be most easily studied is the blood platelet 5-HT, which is taken up by platelets against a concentration gradient. Pletscher & Bernstein (1958) have shown that iproniazid raises the 5-HT content of the platelets of rabbits and of man receiving it in therapeutic dosage. Imipramine, not a monoamine oxidase inhibitor, caused a fall in the platelet 5-HT level of patients on therapy (Marshall, Stirling, Tait & Todrick, 1960). Since the uptake of 5-HT by platelets *in vitro* can also be inhibited by this compound (Marshall & others, 1960; Stacey, 1961; Long & Lessin, 1962), it has been suggested that the fall in 5-HT during therapy is due to inhibition of uptake rather than to interference with 5-HT production.

The therapeutic effectiveness of imipramine has led to the clinical trial of structurally similar compounds. We have compared the effect of these compounds and certain of their metabolites (Herrmann, Schindler & Pulver, 1959) on the uptake of 5-HT by human blood platelets *in vitro*. The effects of chlorpromazine and orphenadrine, a drug with antidepressant action probably of a different type (Robinson, 1961), have also been examined.

Experimental

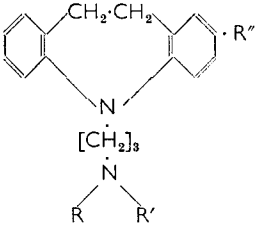
MATERIALS

The compounds investigated were: imipramine, desmethylimipramine (desipramine), 2-hydroxydesmethylimipramine, desdimethylimipramine, amitriptyline, desmethylamitriptyline (nortriptyline), chlorpromazine, orphenadrine.

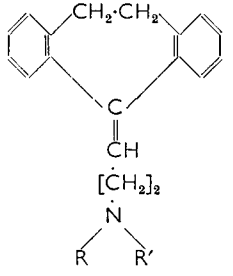
From the Department of Clinical Research, Crichton Royal, Dumfries, Scotland.

* Present address: Department of Pharmacology, University School of Medicine, Edinburgh, 8.

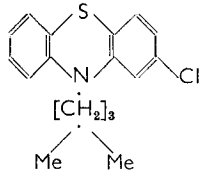
The structures of these compounds are similar, but orphenadrine may have a non-planar configuration resulting from rotation of the benzene rings.



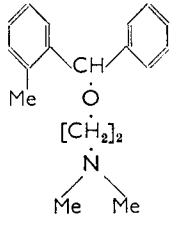
	R	R′	R″
Imipramine	Me	Me	H
Desmethylimipramine	Me	H	H
Desdimethylimipramine	H	H	H
2-Hydroxydesmethyl-imipramine	Me	H	OH



	R	R′
Amitriptyline	Me	Me
Nortriptyline	Me	H



Chlorpromazine



Orphenadrine

The average plasma concentration of "imipramine-like substances" in patients receiving 150 mg desipramine daily was found to be 0·85 μg/ml (Yates, Todrick & Tait, 1963). The effect of the drugs on the uptake of 5-HT by platelets was tested at concentrations of 1 and 4 μg/ml. While it might have been considered preferable to work with equimolar concentrations, the molecular weights of the drugs used are not greatly different. Those of the antidepressant drugs range from 252 to 282 while those of chlorpromazine and orphenadrine are 319 and 268 respectively; the differences in the molar concentrations are therefore comparatively small.

METHODS

20 ml of blood was drawn from normal subjects into 2 ml of a 1% solution of disodium diaminoethanetetracetate in 0·7% saline. The platelet-rich plasma was obtained by centrifugation at 200 *g* for 20 min and the platelets counted by the method of Dacie (1956). The mean of two counts, each by a different operator, on each of two separate dilutions of the platelet-rich plasma, was taken as the platelet count.

Uptake of exogenous 5-HT *by platelets.* The method previously

described (Marshall & others, 1960) was modified as follows:

(*a*) 1·25 ml platelet-rich plasma was used instead of 1·5 ml; this provided six instead of four aliquots and allowed two drugs to be tested simultaneously on the same platelets.

(*b*) Since only a small proportion of added 5-HT was removed by the platelets, additional steps were taken to prevent contamination of the platelet button by the plasma. After incubation, the tubes were chilled in ice-water to stop further uptake, diluted with 8 ml chilled saline, centrifuged at 2000 *g* for 15 min and the supernatant discarded. Any trace of diluted plasma adhering to the tube walls was then washed down with a few drops of saline and the tube recentrifuged at 400 *g* for 5 min. The saline was poured out and the residual liquid removed from the walls with filter paper. The platelet button was suspended in 5 ml saline and the 5-HT estimated in duplicate fluorimetrically as described previously (Yates & others, 1963).

The 5-HT estimates from control tubes to which saline and drug but no 5-HT had been added did not differ significantly from those to which saline alone was added; this confirms that the drugs did not interfere with the 5-HT assay, and also indicates that the drugs at the concentrations used did not release 5-HT from the platelets.

Results

The mean endogenous 5-HT level of the platelet-rich plasma was 46 ng/10^8 platelets. The platelets took up 3·5 $\pm$ 1·3 (s.d.) times their endogenous content of 5-HT.

The data for the effect of the drugs on the uptake of 5-HT by platelets are given in Table 1.

TABLE 1. INHIBITION BY ANTIDEPRESSANT DRUGS AND METABOLITES OF 5-HT UPTAKE BY PLATELETS

(5-HT concentration, 1 μg/ml free base)

Drug concentration (hydrochloride)	1 μg/ml	4 μg/ml
Drug	Percentage inhibition $\pm$ s.d. (mean of 6 estimates)	
Imipramine	54 $\pm$ 5·5	79 $\pm$ 5·8
Desipramine	30 $\pm$ 4·2	56 $\pm$ 9·5
Desdimethylimipramine	32 $\pm$ 5·0	55 $\pm$ 7·1
2-Hydroxydesmethylimipramine	30 $\pm$ 16·0	56 $\pm$ 6·1
Amitriptyline	39 $\pm$ 3·5	68 $\pm$ 6·5
Nortriptyline	24 $\pm$ 4·2	59 $\pm$ 6·8
Chlorpromazine	−6 $\pm$ 5·0	24 $\pm$ 7·8
Orphenadrine	−3 $\pm$ 12·0	−1 $\pm$ 15·4

Imipramine was the strongest inhibitor with amitriptyline next, ($P < 0{\cdot}001$ and $< 0{\cdot}02$ at 1 and 4 μg/ml respectively). They were each more effective than their demethylated derivatives. The three metabolites of imipramine did not differ significantly in potency. Chlorpromazine had no effect at 1 μg/ml but did have an effect at 4 μg/ml. Orphenadrine had no effect at either concentration.

Discussion

The results presented agree qualitatively with those of Stacey (1961) and Long & Lessin (1962), who found that imipramine was a more powerful inhibitor of the uptake of 5-HT by platelets than chlorpromazine. But we found the concentration of imipramine causing 50% inhibition to be about 3×10^{-6} M in contrast to $0{\cdot}5 \times 10^{-6}$ and $0{\cdot}8 \times 10^{-6}$ M obtained by the other two groups of workers. Stacey's technique, used also by Long & Lessin, differs from ours in that (i) the drug is pre-incubated with the platelets for 10 min at 37° before adding 5-HT, (ii) the mixture is gassed with 95% O_2 and 5% CO_2 and (iii) the incubation with 5-HT only lasts 20 min. We found none of these factors contributed to the difference.

Variations in degree of inhibition *in vitro* and of rate of fall of platelet 5-HT *in vivo* might perhaps be expected to correlate with observations of differing speed in clinical action; if this were so, our observations would tend to parallel the more conservative estimates of the clinical effectiveness of desipramine reported by Dick (1961) and Oltman & Friedman (1962).

Our results, together with those of Stacey (1961), indicate that, as a group, the cyclodibenzyls are the most potent inhibitors of the uptake of 5-HT by platelets by an active transport mechanism and produce effects at concentrations obtained *in vivo* during therapy. Comparison between the cyclodibenzyls suggests a small but significant superiority of the parent substances over the demethylated derivatives, paralleling the pharmacological results of Sigg, Soffer & Gyermek (1963) with 5-HT rather than those of Brodie, Bickel & Sulser (1961), Garattini, Giachetti, Jori, Pieri & Valzelli (1962) and Sulser, Watts & Brodie (1962).

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