The binding of imipramine to the outer membrane of blood platelets

SIR,—Imipramine has long been known to inhibit the uptake of noradrenaline into sympathetic nerve endings and 5-hydroxytryptamine (5-HT) into platelets (Marshall, Stirling & others, 1960; Stacey, 1961). It has been assumed that this action is brought about by inhibition of active transport at the outer cell membrane (Carlsson, 1966), however, direct evidence is lacking. We now present experimental evidence that [^{14}C]imipramine is rapidly accumulated at the outer membrane of the human blood platelet.

In two experiments platelets were incubated in 2×10^{-6} or 2×10^{-5}M [^{14}C]imipramine HCl (specific activity 25·4 μc/mg; Radiochemical Centre, Amersham) or 2×10^{-5}M [^{14}C]inulin (specific activity 3·08 μc/mg; New England Nuclear Corporation, Boston) for 1–60 min. The radioactivity in platelets and plasma was estimated by liquid scintillation spectrometry.

The inulin experiments determined the amount of drug which was trapped in the space between the platelets during separation from plasma by centrifugation, and a value of 0·57 μl/μl packed platelets was obtained after 10 or 60 min incubation. This figure is at the upper end of the range found by Born & Gillson (1959).

When comparable experiments were made with imipramine the "apparent" extracellular space was almost exactly 10 times greater (5·80 μl/μl packed platelets). As inulin is commonly used to estimate extracellular space, we propose that the difference between the values obtained with imipramine and inulin represents the binding of the former to the platelets. Therefore when calculating the extent of imipramine binding to platelets we have estimated the percentage of substance localized in or on the cells by subtraction of the proportion of drug trapped between the platelets as predicted from the inulin determinations. The results (Table 1), show that imipramine binds to platelets extremely rapidly with no further increase in accumulation upon prolonged incubation. As the time-course of binding reaches equilibrium within 1 min it is unlikely that imipramine enters the cells. This view is supported by results obtained when four samples of platelet-rich plasma were incubated with 2×10^{-6}M imipramine for 10 and 60 min and the labelled platelets subsequently washed with drug-free plasma. We found that the amount of imipramine retained was reduced from 8 to 1%.

TABLE 1. DISTRIBUTION OF IMIPRAMINE AND INULIN IN PLATELETS

Time of incubation (min)	Imipramine recovered (%)	Inulin recovered (%)	Imipramine bound (%)
1	8·22	0·78	7·44
10	7·36	0·58	6·78
60	8·86	0·55	8·31

Percentage figures refer to the proportion of radioactivity recovered in the platelets separated from 1 ml of platelet-rich-plasma by centrifugation at 2,000 $\times$ *g* for 5 min.
Each result is the mean of 2 values obtained in separate experiments.

Thus, the uptake of imipramine by platelets cannot be explained solely on the basis that the drug is confined to the interstices between the packed cells. The fact that the accumulation is almost instantaneous and that even after incubation for 1 hr, imipramine is easily displaced by washing, supports the belief that imipramine interferes with platelet and sympathetic function by an action on the outer membrane.

Mills & Roberts (1967) have clearly shown that imipramine is a potent inhibitor of platelet aggregation when studied under conditions similar to

those described here. It is probable that their results can be explained on the basis that imipramine, in binding to the cell membrane, prevents the access to the interior of the cell of compounds which cause aggregation, as well as inhibiting the release of ADP from the platelets as they suggested.

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β-Blocking agents on the pupil of the frog

SIR,—It is believed that the sympathetic effect on the dilator pupillae is mediated via α-receptors (Beaver & Riker, 1962; Ahlquist, 1966). The intravenous administration of α-blocking agents like phenoxybenzamine to dogs or rabbits, followed by an intraoccular injection of (−)-adrenaline, (−)-noradrenaline and (−)-isoprenaline inhibits mydriasis. Pretreatment with a β-blocking agent like dichloroisoprenaline in a similar way does not inhibit mydriasis (Bennett, Reinke & others, 1961). When the effect of applied catecholamines is observed on the isolated eye of the frog prepared according to Ehrmann (1905), evidence of the existence for β-receptors is uncovered.

One eye is placed in an isotonic saline solution and serves as a control. The other is placed in isotonic saline solution in which the drug has been dissolved. Propanolol in a concentration of 2 to 5 $\times$ 10^{-5} provokes miosis within 3 hr which 8–12 hr later develops into a complete closure of the pupil; while isoprenaline provokes mydriasis in a concentration of 0·5 to 1 $\times$ 10^{-5}. This latter action is partially inhibited in eyes which have been placed in the propranolol solution.

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