Interference by mepacrine with the storage of 5-hydroxytryptamine in blood platelets

Subcellular distribution experiments with blood platelets have demonstrated that basic compounds like mepacrine and acridine orange selectively accumulate in the 5-hydroxytryptamine (5-HT) storage organelles (Da Prada & Pletscher, 1975). Mepacrine also inhibits the uptake of 5-HT by blood platelets, and another basic substance, methylene blue, liberates this amine from the platelets (Bridges & Baldini, 1966; Feer, 1968; Schick & Yu, 1973). Therefore, polycyclic basic compounds might be used as tools to study the mechanism of storage of 5-HT (and possibly other amines) and, in this context, investigations on their action on platelet 5-HT seemed to be of interest.

The present work deals with the effect of mepacrine on the uptake and liberation of 5-HT in platelets of guinea-pigs (300–400 g), fasted for 16 h. The platelets were isolated as previously described and resuspended in a volume of Tyrode buffer (pH 7·4, devoid of $CaCl_2$ but containing 0·002 M disodiumethylenediamine tetraacetate) corresponding to the original plasma volume (Da Prada, Pletscher & Bartholini, 1965). Part of the platelet suspension (liberation experiments) was preincubated in Tyrode for 30 min at 37° with $0{\cdot}57 \times 10^{-6}$M ^{14}C–5-HT (5-hydroxytryptamine[3-^{14}C]-creatinine sulphate, 55 mCi mmol^{-1}, The Radiochemical Centre, Amersham), centrifuged (1200 *g* for 15 min), washed once and resuspended in Tyrode. The labelled platelets were then incubated for 30 min at 37° in the presence or absence (controls) of various concentrations of mepacrine (K & K Laboratories Inc., Plainview, N.Y., U.S.A.) followed by cooling in ice water and sedimentation by centrifugation.

In other experiments (uptake experiments), platelets suspended in Tyrode were incubated for 15 min at 37° in the presence or absence of various concentrations of mepacrine. ^{14}C–5-HT ($0{\cdot}57 \times 10^{-6}$M) was added to the suspension and the incubation continued for another 30 min followed by cooling and centrifugation, as indicated above.

Finally (metabolic experiments) aliquots of platelet suspensions were pre-incubated in Tyrode for 30 min at 37° in the presence or absence of 10^{-5}M mepacrine, washed once and reincubated for 2 h with 10^{-6}M ^{14}C–5-HT. After sedimentation and one washing (Tyrode), the ^{14}C–5-HT content of the platelets and the amount of [^{14}C]5-hydroxyindoleacetic acid (5-HIAA) in the incubation medium were determined.

For the measurement of ^{14}C–5-HT, the platelet pellets were lysed in 1% disodium-dodecyl sulphate and after addition of the lysate to 10 ml Instagel counted for radioactivity in a Packard Tricarb spectrometer. The counting efficiency (75%) was determined using the double channel ratio. The ^{14}C–5-HIAA of the incubation medium (plus washing) was extracted as previously described (Da Prada & others, 1965) and counted for radioactivity after adding the final extracts to 10 ml Instagel. Proteins were measured according to Lowry, Rosebrough & others (1951). All centrifugations of platelets were at +4°.

Mepacrine, in concentrations between 10^{-6} and 10^{-4}M, progressively decreased the labelled 5-HT stored in the platelets indicating a liberation of the amine. The drug also diminished the uptake of ^{14}C–5-HT by the platelets. The percentage decrease of the stored ^{14}C–5-HT and of the ^{14}C–5-HT uptake did not significantly differ at the mepacrine concentrations used (Fig. 1). Furthermore, preliminary experiments indicated that mepacrine diminished the endogenous 5-HT to the same extent as the stored labelled amine.

With regard to the mode of action of mepacrine, a non-specific damage of the platelets is unlikely at least with the lower concentrations of the drug. Thus, based on previous electron microscopic studies (Picotti, Da Prada & Pletscher, 1975)

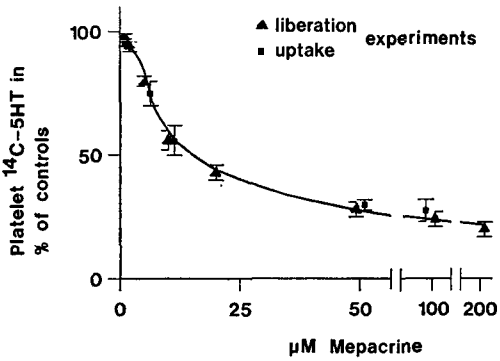

FIG. 1. Effect of various concentrations of mepacrine on the uptake and liberation of [^{14}C]5-hydroxytryptamine (^{14}C–5-HT) in isolated platelets of guinea-pigs. Each point represents an average with s.e. of 3 (uptake) and 4–6 (liberation) experiments, respectively, and indicates the ^{14}C–5-HT content of platelets in % of controls (=100). Absolute ^{14}C–5-HT values of control platelets in nmol mg^{-1} protein: uptake experiments: 0·56 ± 0·05, n = 10; liberation experiments: 0·54 ± 0·01, n = 10.

platelets incubated with mepacrine up to 10^{-5}M (10 μM) remained intact, whereas with 10^{-4}M of the drug part of them (about 20%) showed rupture of the cytoplasmatic membrane and extracellular location of cytoplasmatic constituents. Experiments in which the formation of 5-HIAA was measured supplied information as to the site of action of mepacrine. Thus, the drug in a concentration which markedly reduced the ^{14}C–5-HT uptake by platelets increased the content of ^{14}C–5-HIAA in the incubation medium (Table 1). This indicates that the drug interferes with the intracellular 5-HT storage and not with the transport at the cytoplasmatic membrane. As previously shown (Pletscher, Burkard & others, 1967), inhibition of the ^{14}C–5-HT uptake at the cytoplasmatic membrane (e.g. by imipramine) decreases the formation of the ^{14}C–5-HT metabolite ^{14}C–5-HIAA owing to a diminished access of the amine to the intracellular metabolic sites (e.g. mitochondria). In contrast, drugs (e.g. reserpine) interfering with the intracellular storage of 5-HT increase the formation of 5-HIAA since the amine taken up through the cytoplasmatic membrane is no longer protected from metabolic enzymes by accumulation in storage organelles. An action of mepacrine at the level of the 5-HT storage organelles also agrees with the finding that the drug is equally potent with regard to inhibition of uptake and liberation of 5-HT (Fig. 1).

Thus, mepacrine shows a pattern of action similar to that of reserpine (Pletscher & others, 1967), although differences in the mechanism of action of the two drugs seem to exist. For instance, the stoichiometric relation between drugs taken up by the storage organelles and the 5-HT liberated from the organelles differs for the two drugs. In fact, 1 molecule of reserpine liberates more than 100 molecules of 5-HT, whereas about 30 molecules of mepacrine have to be taken up by the platelets for the liberation of 1 molecule 5-HT (Picotti, Da Prada & Pletscher, in preparation; Carlsson, Shore & Brodie, 1957).

Preliminary experiments indicate that a variety of basic colourants (including the lipid insoluble methylene blue, see above), but not acidic dyes, liberate 5-HT from platelets. It is possible that the basic compounds interact with acid constituents either of the membranes (phospholipids, sulphatides, lipoproteins) or of the interior (adenosine-5′-triphosphate) of the storage organelles and thereby change their physicochemical properties leading to a decreased storage of 5-HT in the organelles.

Table 1. *Effect of mepacrine on [^{14}C]5-hydroxytryptamine (^{14}C-5-HT) uptake and formation of [^{14}C]5-hydroxyindoleacetic acid (5-HIAA) in isolated platelets of guinea-pigs.* Averages with s.e.m. of 3 experiments performed on 3 different days. The absolute values (pmol mg^{-1} platelet protein) were related to the control values of the same day (% values).

Incubation with	^{14}C-5-HT uptake pmol mg^{-1} protein	%	^{14}C-5-HIAA formation pmol mg^{-1} protein	%
Controls	586·7 ± 112·6	100	26·3 ± 4·1	100
Mepacrine	376·7 ± 96·2	62·33 ± 7·15	55·0 ± 8·1	209·2 ± 1·6

Mepacrine and methylene blue (a weak inhibitor of monoamine oxidase) also inhibit the uptake of ^{3}H-5-HT and [^{3}H]dopamine, respectively, in crude synaptosomal fractions of rat forebrain and caudate nucleus (Carruba & Picotti, personal communication). Therefore, the possibility has to be considered that an interference with amine storage and/or metabolism in brain explains the occasional observations of psychogenic disturbances in man, e.g. sexual disorders and mental confusion induced by mepacrine and methylene blue, respectively (Goodman & Gilman, 1956; Kapur & Gupta, 1950; Schick & Yu, 1973).

In conclusion, the liberation of 5-HT as well as the inhibition of its uptake in platelets by basic substances such as mepacrine is probably due to their action at the level of the subcellular 5-HT storage organelles. Therefore, these compounds, some of which (e.g. mepacrine) can be visualized in the 5-HT storage organelles of live platelets by fluorescence microscopy (Lorez, Da Prada & Pletscher, 1975), may be useful tools for studying the mechanism of intracellular amine storage.

Institute of Pharmacology,
School of Medicine,
University of Milan, Italy

G. B. PICOTTI

Research Division,
F. Hoffmann-La Roche & Co. Ltd.,
Basle, Switzerland

M. DA PRADA
A. PLETSCHER

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