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5-HYDROXYTRYPTAMINE AND DOPAMINE TRANSPORT BY RAT AND HUMAN BLOOD PLATELETS

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1 Uptake of 5-hydroxytryptamine (5-HT) by rat platelets in plasma was very rapid and diffusion did not contribute significantly at substrate concentrations that did not saturate the active transport.

2 Under conditions which allowed measurement of initial rates of uptake, kinetic analysis revealed a high affinity uptake mechanism for 5-HT ($K_m = 0.7\ \mu M$).

3 Uptake of dopamine was relatively slow and involved a lower affinity ($K_m = 70\ \mu M$) active transport process. Diffusion contributed significantly at concentrations that did not saturate the active transport.

4 5-HT competitively inhibited uptake of dopamine, and *vice versa*; K_i values for both amines were similar to their respective K_m values for uptake.

5 Chlorimipramine, desmethylimipramine and benztropine were tested as uptake inhibitors. Each was equipotent against 5-HT and dopamine, although the absolute potency of the drugs varied greatly. Chlorimipramine was the most potent ($K_i \simeq 100$ nM), and kinetic analysis revealed that the inhibition was competitive against both 5-HT and dopamine.

6 Similar results were obtained in studies with human platelets: K_m values for 5-HT and dopamine were about $1\ \mu M$ and $100\ \mu M$ respectively. Activity profiles of inhibitors were also similar: each compound tested was equipotent against 5-HT and dopamine, and the two amines each competitively inhibited uptake of the other.

7 We conclude that dopamine is actively transported by platelets via the 5-HT uptake mechanism, but with a much lower affinity. There is no high-affinity dopamine-specific mechanism corresponding to that in the corpus striatum. Consequently although platelets may be valid models of transport in 5-hydroxytryptaminergic neurones, they should not be regarded as models for the dopamine transport mechanism found in dopaminergic neurones.

Introduction

Platelets from many species transport 5-hydroxytryptamine (5-HT) (Born & Gillson, 1959; Stacey, 1961; Tuomisto, 1974; Drummond & Gordon, 1976) and store it in granules as a macromolecular complex with adenosine triphosphate (ATP) and calcium (Da Prada & Pletscher, 1968). If initial rates of uptake are measured, the kinetics and pharmacology of the transport mechanism closely resemble the uptake of 5-HT by brain synaptosomes (Tuomisto, 1974). Human platelets also accumulate dopamine against a concentration gradient by a mechanism that is energy-dependent and temperature-sensitive (Boullin & O'Brien, 1970; Solomon, Spirt & Abrams, 1970), but the kinetics and pharmacology of this process are less well characterized than those for 5-HT.

Platelets have been proposed as models for the study of aminergic neurones (see, for example: Abrams & Solomon, 1969) but there has been some debate as to whether this is valid for the dopaminergic neurone (Sneddon, 1973; Trenchard, Turner, Pare & Hills, 1975), partly because it was not clear whether the uptake of dopamine by dopaminergic neurones in the corpus striatum (Snyder & Coyle, 1969; Horn, Coyle & Snyder, 1971) occurred by a mechanism analogous to that in platelets.

Most of the work on uptake by aminergic neurones has been carried out on preparations of rat brain. We have therefore compared, in detail, the uptake of 5-HT and dopamine by rat platelets, with particular regard to the kinetics and pharmacology of the

processes, in order to determine whether platelets may be suitable models for studying the uptake mechanism in dopaminergic as well as 5-hydroxytryptaminergic neurones. Less detailed studies have also been performed with human platelets.

Methods

Preparation of platelet-rich plasma (PRP)

Rat blood was obtained by venepuncture of the inferior vena cava, from male animals weighing 300 to 400 g anaesthetized with ether. Samples were anticoagulated with trisodium citrate (final concentration in blood 3.1 mg/ml), and centrifuged at room temperature for 2 min at 2250 *g*. Human citrated blood samples, obtained by antecubital venepuncture from volunteers who had allegedly taken no medication for at least 10 days, were centrifuged for 60 s at 2250 *g*. The supernatant PRP was removed and the platelet count determined with a Coulter Thrombo-counter. For rat experiments, PRP samples from 2 to 5 animals were pooled. Reference to triplicate or quadruplicate determinations (see Figures) means replicates from such a sample. Where mean values for K_m and V_{max} are quoted, *n* refers to the number of separate experiments performed.

Uptake of [^{3}H]-5-hydroxytryptamine and [^{3}H]-dopamine

Uptake of 5-HT and dopamine was measured by incubating 0.1 ml or 0.2 ml samples of PRP at 37°C with various concentrations of 5-hydroxy-[^{3}H]-tryptamine ([^{3}H]-5-HT) or [^{3}H]-dopamine respectively. PRP samples were preincubated for 2 min at 37°C with drug or 0.9% w/v NaCl solution (saline) before adding the [^{3}H]-amine. Uptake of the [^{3}H]-amine was terminated by the addition of five volumes of ice-cold iso-osmotic saline containing 0.4% w/v disodium edetate (EDTA-saline); samples were immediately centrifuged (30 s; 14,700 *g*) in a Quickfit microcentrifuge. Replicate samples were included in which the [^{3}H]-amine was added after the PRP was cooled to 4°C; this allowed the results to be corrected for radioactivity trapped in the cell pellets or adhering to the tubes. The supernatants were decanted and the platelet pellets washed with 1.0 ml ice-cold EDTA-saline. The pellets were digested with 0.2 ml formic acid (21 M) for 1 h at room temperature, then transferred to plastic mini-vials containing 5 ml of scintillant (toluene with 0.33% w/v 5-(4-biphenylyl)-2-(4-*t*-butylphenyl)-1-oxa, 3,4 diazole (butyl PBD) plus 30% v/v 2-ethoxyethanol). Radioactivity was measured in a Nuclear Chicago Mk 2 liquid scintillation counter. In some experiments uptake of [^{3}H]-5-HT was also measured as the decrease in supernatant radioactivity after separation of platelets and plasma by centrifugation. Platelets of all species tested (including man) metabolize 5-HT and dopamine very slowly: more than 90% of the radioactivity in the platelet represents unmetabolized amine (Stacey, 1961; Pletscher, 1968; Solomon *et al.*, 1970; Sneddon, 1973).

Materials

5-Hydroxy-[G-^{3}H]-tryptamine creatinine sulphate (0.5 Ci/mmol; The Radiochemical Centre, Amersham, Bucks) was dissolved in iso-osmotic saline to a concentration of 10^{-4} M and stored in 0.25 ml volumes at +4°C. 3,4-Dihydroxy-[ring-G-^{3}H]-phenylethylamine hydrochloride ([^{3}H]-dopamine; 8.5 Ci/mmol; The Radiochemical Centre) was dissolved in iso-osmotic saline to a concentration of 5×10^{-4} M, and the specific activity adjusted to 0.8 Ci/mmol with unlabelled dopamine. Sodium metabisulphite (final concentration 0.05%) was added as an antioxidant. Samples (50 µl) of [^{3}H]-dopamine were sealed under nitrogen and stored in liquid nitrogen. The concentrations of [^{3}H]-5-HT and [^{3}H]-dopamine required were obtained by dilution with iso-osmotic saline or addition of unlabelled amine immediately before use.

Results

Time course of uptake of [^{3}H]-5-hydroxytryptamine and [^{3}H]-dopamine into rat platelets

When 5-HT, initial substrate concentration 1 µM, was incubated with PRP, uptake was linear for at most 10 s and was largely complete within 1 min (Figure 1a). The termination of uptake was due at least in part to substrate depletion: after 10 s and 60 s the 5-HT plasma concentrations were about 0.8 µM and 0.5 µM respectively. This substrate depletion could be largely overcome by dilution of the PRP with cell-free plasma, and thus reducing the platelet concentration. Although the linear component could be prolonged by further dilution of the PRP, the practical difficulties associated with the need for large volumes of rat plasma made this undesirable.

At comparable substrate concentrations uptake of dopamine by rat platelets was much slower than uptake of 5-HT. Uptake of [^{3}H]-dopamine was approximately linear for several minutes at a substrate concentration of 1 µM, and there was less than 5% substrate depletion after 5 min incubation with 1 µM dopamine, even when undiluted PRP was used (Figure 1b).

Kinetics of uptake of [^{3}H]-5-hydroxytryptamine into rat platelets

Uptake of 5-HT by platelets is known to take place both by active transport and diffusion (Pletscher,

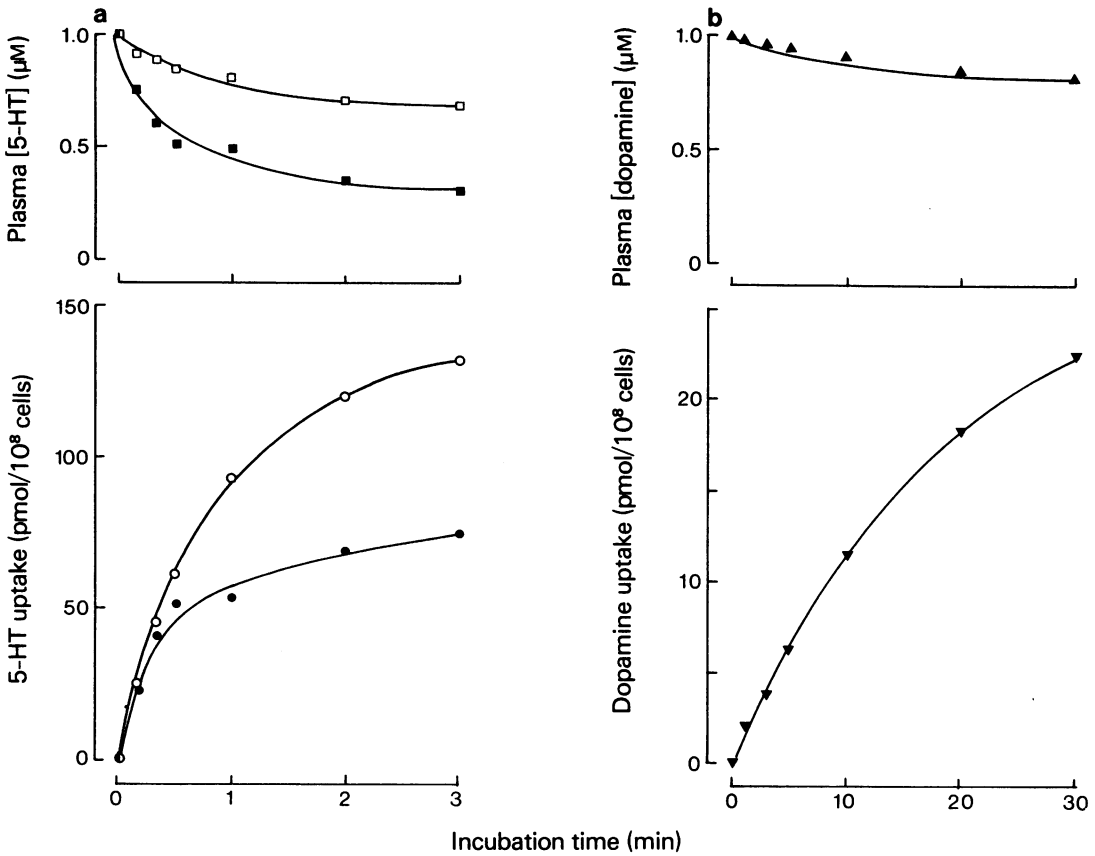

Figure 1 Rate of uptake of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) and [^{3}H]-dopamine by rat platelets. (a) Samples (0.1 ml) of PRP (10^9 cells/ml) or PRP diluted with cell-free plasma (2.5×10^8 cells/ml) were incubated at 37°C with [^{3}H]-5-HT (1 μM). At the times indicated, 0.5 ml of ice cold EDTA-saline was added and samples were centrifuged (14,700 *g*; 30 s). Radioactivity in the cell pellets from PRP (●) and diluted PRP (○), and the radioactivity remaining in the supernatants from PRP (■) and diluted PRP (□) were determined as described in the Methods section. Each point is the mean of triplicate determinations. (b) Samples (0.2 ml) of PRP were incubated at 37°C with [^{3}H]-dopamine (1 μM) and at the times indicated radioactivity in the cells (▼) and in the supernatant (▲) was determined as described for [^{3}H]-5-HT. Each point is the mean of quadruplicate determinations.

1968). Results of preliminary experiments indicated that the 5-HT entering by diffusion could not be measured accurately for short incubation times. Therefore, in order to estimate the contribution made by diffusion, samples of PRP (diluted to 3×10^8 cells/ml) were incubated with [^{3}H]-5-HT (0.3 to 100 μM) for 5 min at 37°C in the presence and absence of 50 μM chlorimipramine, which is a potent competitive inhibitor of the active transport of 5-HT.

When uptake was plotted against 5-HT concentration under these experimental conditions, a two phase curve was obtained in control samples (Figure 2). The value for the non-saturable component was 60 pmol 10^{-8} cells min^{-1} at 100 μM 5-HT. Chlorimipramine abolished the saturable component, but did not affect the non-saturable component (Figure 2). It should be emphasized that because an incubation time of 5 min was used in these experiments, accurate kinetic constants for the saturable transport cannot be derived. The non-saturable uptake of [^{3}H]-5-HT was unaffected by all inhibitors of active transport that we tested and exhibits the same characteristics as the process previously described as 'diffusion' or 'passive transfer' by several other investigators (Fuks, Lanman & Schanker, 1964; Pletscher, 1968; Sneddon, 1973; Tuomisto, 1974). At low 5-HT concentrations (<5 μM) diffusion contributed little to the total uptake, and when uptake is measured after 10 s incubation, rather than 5 min, this contribution is insignificant; for example, it was calculated as 0.1 pmol

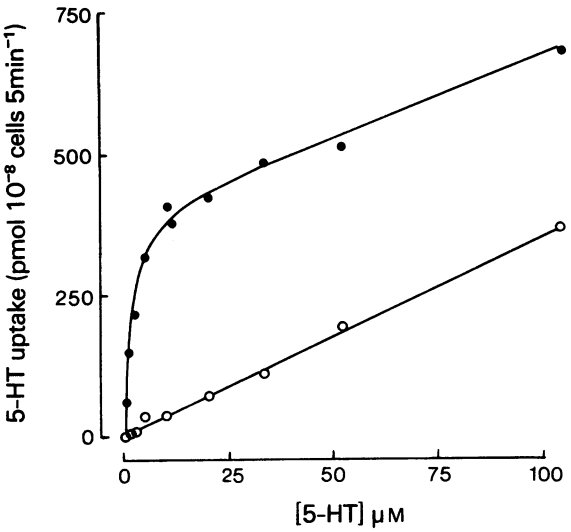

Figure 2 Concentration dependence of the uptake of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) by rat platelets. PRP was preincubated at 37°C with iso-osmotic saline (●) or chlorimipramine 50 μM(○) for 2 min before addition of [^{3}H]-5-HT (0.3 to 100 μM). Uptake of [^{3}H]-5-HT was measured after 5 min incubation at 37°C as described in the Methods section. Each point is the mean of quadruplicate determinations.

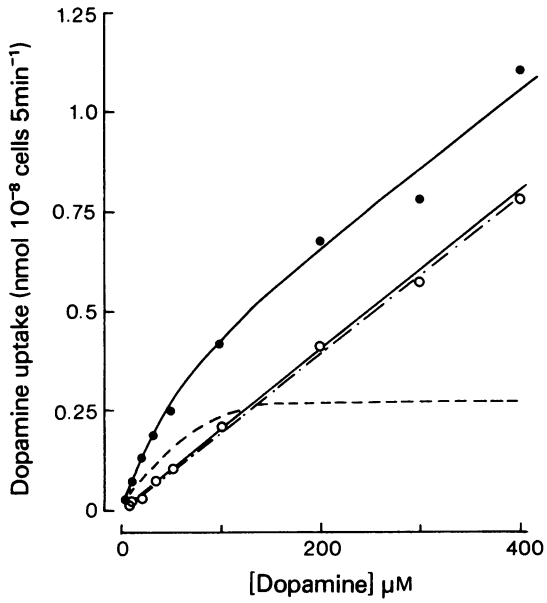

Figure 3 Concentration-dependence of the uptake of [^{3}H]-dopamine by rat platelets. PRP was pre-incubated at 37°C with iso-osmotic saline (●) or an equimolar concentration of 5-hydroxytryptamine (5-HT, ○) for 2 min before addition of [^{3}H]-dopamine (3 to 400 μM). Uptake of [^{3}H]-dopamine was determined after 5 min incubation as described in the Methods section. Uptake in control samples could be resolved into a rectilinear component (–•–•–) and a saturable component (- - - - -). Each point is the mean of quadruplicate determinations.

10^{-8} cells $10\,s^{-1}$ compared with about 25 pmol 10^{-8} cells $10\,s^{-1}$ entering by active transport at 1 μM 5-HT.

With an incubation time of 10 s, and substrate concentrations of 0.3 to 2.5 μM, Lineweaver-Burk (1934) analysis revealed that the saturable transport process had a K_m value of 0.72 ± 0.06 μM and a V_{max} of 59 ± 2 pmol 10^{-8} cells $10\,s^{-1}$ (mean values ±s.e.; $n = 9$). This process was competitively inhibited by chlorimipramine with a K_i value of about 75 nM.

Kinetics of uptake of [^{3}H]-dopamine into rat platelets

As with 5-HT, a biphasic curve was obtained when uptake of [^{3}H]-dopamine (3 to 400 μM) was plotted against substrate concentration. This curve could be resolved into a saturable and a non-saturable component (Figure 3). The non-saturable component had a value of 40 pmol 10^{-8} cells min^{-1} at 100 μM dopamine. Lineweaver-Burk (1934) analysis of the saturable uptake of dopamine gave values of 72 ± 4.3 μM for the K_m and 85 ± 7.4 pmol 10^{-8} cells min^{-1} for V_{max} (mean values ± s.e.; $n = 5$).

It is clear from these results that, in contrast to the uptake of 5-HT by rat platelets, non-saturable uptake contributed significantly to the total uptake of dopamine even at concentrations that did not saturate the active transport process. This non-saturable uptake was unaffected by all the inhibitors tested that abolished the saturable uptake (q.v.), and is apparently a process similar to the 'diffusion' component of 5-HT uptake.

Inhibition by 5-hydroxytryptamine of uptake of [^{3}H]-dopamine into rat platelets

When PRP samples were incubated for 2 min with an equimolar concentration of 5-HT before addition of [^{3}H]-dopamine, the active uptake of dopamine was abolished, but diffusion was unaffected (Figure 3). In further experiments, designed to study the effects of potential inhibitors, diffusion was determined in replicate samples by measuring that part of dopamine uptake insensitive to inhibition by equimolar 5-HT. This component was subtracted from the total uptake, which allowed the potency of inhibitors of the active uptake of dopamine to be more accurately determined.

5-HT and dopamine are both transported into the dense storage granules of platelets (Da Prada &

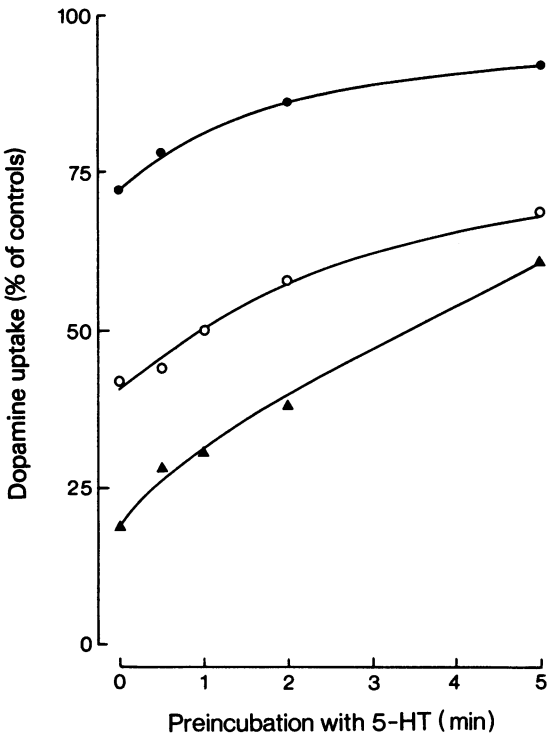

Figure 4 Inhibition by 5-hydroxytryptamine (5-HT) of [^{3}H]-dopamine uptake by rat platelets: effect of preincubation with 5-HT. PRP was preincubated at 37°C for up to 5 min with 5-HT 1 μM (●); 2 μM (○) or 3 μM (▲). Uptake of [^{3}H]-dopamine (20 μM) was then determined as described in the text. Each point is the mean of triplicate determinations. Dopamine uptake in the presence of 5-HT was calculated as a percentage of that in saline controls; inhibition of uptake by each concentration of 5-HT was maximal when 5-HT was added simultaneously with dopamine.

Pletscher, 1969), and we therefore wished to determine whether the inhibition of dopamine uptake by 5-HT was due to an effect on the plasma membrane or an intracellular action.

Samples of PRP were preincubated with 5-HT (1 to 3 μM) for 0 to 5 min before addition of [^{3}H]-dopamine 20 μM. Inhibition of dopamine uptake was maximal when the dopamine and 5-HT were added simultaneously (Figure 4), which suggests that 5-HT exerts its inhibitory effect primarily at the platelet plasma membrane. The decrease in inhibition as 5-HT was preincubated is consistent with the rapid uptake of 5-HT shown in Figure 1. Kinetic analysis of the inhibition, using diluted PRP, showed that it was competitive with a K_i value for 5-HT of about 1 μM.

The corollary of this competitive inhibition (viz. the effect of dopamine on 5-HT uptake) was also investigated. Dopamine inhibited 5-HT uptake, and kinetic analysis (Lineweaver & Burk, 1934; Dixon, 1953) showed this inhibition to be competitive, with a K_i value for dopamine of about 85 μM.

Inhibition of 5-hydroxytryptamine and dopamine uptake into rat platelets

Several drugs were tested as potential inhibitors of 5-HT and dopamine uptake by rat platelets. Figure 5 shows the inhibitory effects of chlorimipramine, desmethylimipramine and benztropine, which are respectively selective inhibitors of amine uptake into 5-hydroxytryptaminergic, noradrenergic and dopaminergic neurones in the central nervous system (Horn *et al.*, 1971; Horn, 1976). Each drug was virtually equipotent against the uptake of 5-HT and dopamine, although the absolute potency of the drugs varied greatly. Inhibition by all three agents was competitive; K_i values were about 0.09 μM for chlorimipramine, 2 μM for desmethylimipramine and 40 μM for benztropine.

Uptake of 5-hydroxytryptamine and dopamine by human platelets

The characteristics of 5-HT uptake by human platelets are well established (Born & Gillson, 1959; Stacey, 1961; Todrick & Tait, 1969; Gordon & Olverman, 1976; Wielosz, Salmona, de Gaetano & Garattini, 1976b). When initial rates of uptake were measured, kinetic analysis of the active uptake revealed that the process had a K_m value of 0.9 ± 0.07 μM and a V_{max} of 28 ± 3.2 pmol 10^{-8} cells $10\,s^{-1}$ (mean values ±s.e.; $n = 8$).

The uptake of dopamine by human platelets was similar to the uptake by rat platelets. Uptake was linear for at least 5 min; a biphasic curve was obtained when uptake of [^{3}H]-dopamine (25 to 450 μM) was plotted against substrate concentration. Only the saturable uptake was inhibited by 5-HT (Figure 6). The non-saturable component had a value of 90 pmol 10^{-8} cells min^{-1} at 100 μM dopamine. Lineweaver-Burk (1934) analysis of the saturable uptake of dopamine gave values of 108 ± 12 μM for the K_m and 176 ± 14 pmol 10^{-8} cells min^{-1} for V_{max} (mean values ±s.e.; $n = 4$).

As with rat platelets, 5-HT competitively inhibited the saturable uptake of dopamine and *vice versa*; K_i values were 1.3 μM for inhibition of dopamine uptake by 5-HT and 180 μM for inhibition of 5-HT uptake by dopamine. Saturable uptake of dopamine was also blocked by inhibitors of 5-HT uptake, and each inhibitor was approximately equipotent against both amines; for example, the K_i value for chlorimipramine was 20 nM against 5-HT uptake and 37 nM against dopamine uptake.

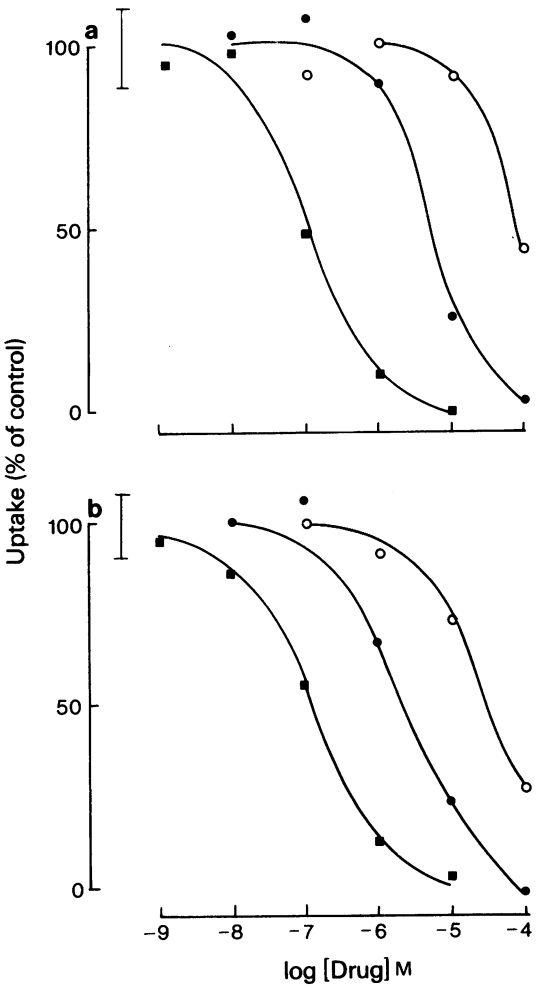

Figure 5 Effect of chlorimipramine (■), desmethylimipramine (●) and benztropine (○) on (a) uptake of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) by rat platelets and (b) uptake of [^{3}H]-dopamine by rat platelets. Samples of PRP were preincubated at 37°C for 2 min with iso-osmotic saline or drug before addition of [^{3}H]-5-HT 0.8 μM or [^{3}H]-dopamine 20 μM. Uptake was determined as described in the text after incubation at 37°C for 10 s (5-HT) or 5 min (dopamine). Results are expressed as a percentage of the uptake in samples without any drug. Each point is the mean of quadruplicate determinations. Vertical bar shows range of control values.

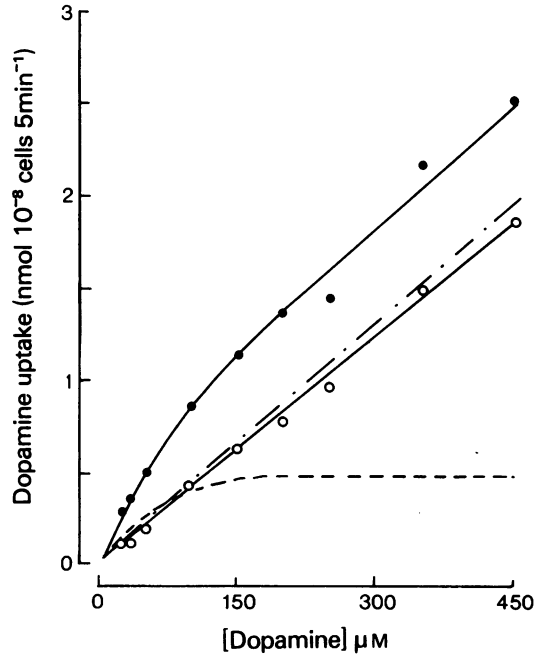

Figure 6 Concentration-dependence of the uptake of [^{3}H]-dopamine by human platelets. PRP was preincubated at 37°C with iso-osmotic saline (●) or a half-equimolar concentration of 5-hydroxytryptamine (5-HT, ○) for 2 min before addition of [^{3}H]-dopamine (25 to 450 μM). Uptake of [^{3}H]-dopamine was determined after 5 min incubation as described in the Methods section. Uptake in control samples could be resolved into a rectilinear component (—•—•—) and a saturable component (- - - - -). Each point is the mean of triplicate determinations.

Discussion

The purpose of this work was to compare the transport processes for 5-HT and dopamine in platelets, and to explore the concept that the platelet is a suitable model for studying the neuronal reuptake of 5-HT and dopamine. Because most studies of neuronal reuptake have used rat brain preparations, our experiments were performed mainly on rat platelets.

Uptake of 5-HT by rat platelets was very rapid, and non-saturable uptake did not contribute significantly at substrate concentrations which did not saturate the active transport. This non-saturable uptake has been referred to as 'passive transfer' or 'diffusion' by previous investigators; despite the fact that it is temperature-sensitive, this conclusion is probably valid, as the process is unaffected by metabolic inhibitors or by drugs that abolish the active transport of 5-HT (Fuks *et al.*, 1964; Pletscher, 1968; Sneddon, 1973; Tuomisto, 1974). To analyse accurately the kinetics of 5-HT uptake the platelet concentration in PRP had to be reduced, thereby minimizing substrate depletion. In addition, incubations with 5-HT were limited to 10 s, and under these conditions the measured rate of uptake was approximately that of the initial rate. If these precautions are not observed the affinity of 5-HT for the uptake mechanism and

the potency of uptake inhibitors may be underestimated (Gordon & Olverman, 1976; Wielosz, de Gaetano & Garattini, 1976a).

The kinetics of 5-HT uptake by rat and human platelets are similar to those found in other studies using comparable experimental conditions (Drummond & Gordon, 1976; Wielosz *et al.*, 1976b) and are also similar to those of the high affinity 5-HT uptake into rat hypothalamic slices (Shaskan & Snyder, 1970) and into synaptosomes (Balfour, 1973).

The uptake of dopamine by rat platelets was relatively slow, and non-saturable uptake contributed significantly even at substrate concentrations which did not saturate the active transport process. The non-saturable uptake process exhibited the same characteristics as the 'diffusion' component of 5-HT uptake. The active transport process had a much lower affinity ($K_m = 70\,\mu M$) than that for 5-HT ($K_m = 0.7\,\mu M$); in contrast, the uptake of dopamine by dopaminergic neurones has a K_m value of $0.13\,\mu M$ (Holz & Coyle, 1974). This strongly suggests that the rat platelet does not possess the high-affinity dopamine-specific transport mechanism found in the corpus striatum.

Dopamine uptake by rat platelets has apparently not been studied before, and although there are several reports of dopamine transport by human platelets (Solomon *et al.*, 1970; Boullin & O'Brien, 1970; Trenchard *et al.*, 1975) the experimental conditions used have not permitted accurate analysis of the kinetics; in particular, the contribution made by diffusion has been neglected. Our results indicate that, as with 5-HT uptake, the characteristics of dopamine uptake by human platelets are broadly similar to those of rat platelets.

Dopamine competitively inhibited uptake of 5-HT, and *vice versa*; also, the K_i value for the inhibiting amine was in each case close to its own K_m value for transport. These results, together with our kinetic data, suggest that dopamine is taken up by platelets via the 5-HT transport process but with a much lower affinity.

This conclusion is strengthened by our observation that benztropine a potent inhibitor of dopamine uptake into dopaminergic neurones (Horn *et al.*, 1971) but a weak inhibitor of 5-HT uptake into 5-hydroxytryptaminergic neurones (Shaskan & Snyder, 1970), was a weak inhibitor of dopamine uptake by platelets and equiactive against 5-HT uptake. Tricyclic antidepressants such as chlorimipramine were potent competitive inhibitors of the active transport of dopamine by platelets and were equiactive against 5-HT. Imipramine and analogues are recognised as being potent inhibitors of 5-HT transport in platelets (Stacey, 1961; Tuomisto, 1974; Drummond & Gordon, 1975; Gordon & Olverman, 1976) and in 5-hydroxytryptaminergic neurones (Shaskan & Snyder, 1970; Horn & Trace, 1974) whereas uptake into dopaminergic neurones is much less affected (Horn *et al.*, 1971). Trenchard *et al.* (1975) found chlorimipramine to be a relatively poor inhibitor of dopamine uptake by platelets but their studies did not take account of dopamine entry by diffusion, which is unaffected by chlorimipramine and which we calculated was responsible for over 50% of their measured uptake at $50\,\mu M$ dopamine.

Our observation that the V_{max} value for 5-HT transport by rat platelets was about 5-fold higher than that for dopamine might seem at first sight to be inconsistent with the concept that the two amines are transported by the same process; however, a common V_{max} value for different substrates sharing the same carrier should be anticipated only when measurements are made under equilibrium conditions, not under steady state conditions, and there are several examples of substrates transported by the same carrier that exhibit different V_{max} values (Christensen, 1975). For example, Shaskan & Snyder (1970) found substantial differences in the V_{max} values for 5-HT and noradrenaline uptake (via the same carrier) into catecholaminergic neurones.

In summary, we have found no evidence to suggest that rat or human platelets possess an active transport process specific for dopamine. The platelet transport process for 5-HT is apparently virtually identical to that in rat brain synaptosomes, and therefore the use of platelets as models of transport in 5-hydroxytryptaminergic neurones seems justified, particularly as they offer several experimental advantages (e.g. they are easily obtained, sequential samples can be taken, and unlike studies on brain preparations there is no interference from other amine uptake mechanisms). In contrast, although human platelets may possess dopamine receptors analogous to those at postsynaptic dopaminergic sites (Boullin, Green & Grimes, 1976), our results with rat and human platelets indicate that they cannot be regarded as models for the amine transport mechanism found in the dopaminergic neurones of the corpus striatum.

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