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FUNCTIONAL DISTINCTION BETWEEN SEROTONIN UPTAKE AND SEROTONIN-INDUCED SHAPE CHANGE RECEPTORS IN RAT PLATELETS

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Tyramine and dopamine are taken up by rat platelets through the serotonin uptake mechanism while phenethylamine is not taken up. This indicates that an aromatic hydroxyl group is a structural requirement for the uptake of phenethylamine derivatives by rat platelets. Although none of these phenethylamine derivatives induce platelet shape change, they inhibit serotonin-induced shape change and serotonin uptake with the same relative potency (tyramine > phenethylamine ⩾ dopamine). This suggests that the receptors controlling serotonin uptake and serotonin-induced shape change have a common structural component that binds phenethylamine derivatives. However, the fact that phenethylamine derivatives activate the serotonin uptake mechanism but do not induce platelet shape change suggests that serotonin uptake and serotonin-induced shape change are mediated by two distinct activation sites of serotonin receptors.

Introduction

Serotonin is taken up by platelets through an energy-dependent transport process (see, for review, Ref. 1) and stimulates platelet shape change and aggregation [2]. There is evidence that these serotonin-platelet interactions are mediated by two distinct platelet receptors. Born and his colleagues [3,4] showed that the structural specificities of the platelet receptors controlling these serotonin-induced actions were quite different. Subsequently Drummond and Gordon [5] and Peters and Grahame-Smith [6] directly demonstrated two distinct binding sites for serotonin on the platelet membrane, whereas Laubscher and Pletscher [7] pharmacologically dissociated the two activities by the use of neuropsychotropic drugs.

It has been reported that tyramine [8] and dopamine [9] are taken up by rat platelets by means of the serotonin transport mechanism. The two compounds are, respectively, the mono and dihydroxylated derivatives of β-phenethylamine. It has been suggested [8] that hydroxylation of benzylic ring of phenethylamine may determine the affinity of the different compounds for serotonin transport system. However, it is not known whether phenethylamine itself is actively transported across rat platelet membrane.

The purpose of the present work was: (1) to compare the relative affinity of tyramine, dopamine and phenethylamine for the serotonin active transport system; (2) to examine whether these three amines induce platelet shape change; (3) to evaluate the possible interaction of the three compounds with both the active uptake of serotonin and the serotonin-induced shape change.

Materials and Methods

Blood was obtained from 250–300 g CD-COBS rats (Charles River, Calco, Italy) as previously described [8] except that 0.103 M trisodium citrate

was used as anticoagulant. Platelet-rich plasma was diluted with autologous platelet-poor plasma to a final platelet count of about 400 000/μl.

Platelet uptake of labeled amines

The uptake of the labeled amines was measured using a modification [8,11] of the method of Gordon and Olverman [9]. The optimal incubation time for accurate and sensitive kinetic analysis of uptake data was determined for each labeled amine from preliminary time-course experiments. Incubations of 10, 15 and 60 s, respectively, were selected for serotonin, tyramine and dopamine. Incubation with phenethylamine ranged from 10 s to 30 min. When the inhibitory effects of drugs or unlabeled amines upon the uptake of labeled amines were studied, platelet-rich plasma samples were preincubated with inhibitors for 2 min at 37°C before addition of the labeled amine.

Platelet shape change

Aliquots of 0.25 ml platelet-rich plasma were preincubated for 2 min at 37°C with 2.5–5 μl of either isotonic saline or test drug at different concentrations. Shape change was induced by adding 2.5–5 μl serotonin (at different concentrations) to the stirred platelet-rich plasma samples in the cuvette of an ELVI 840 aggregometer (Elvi Logos, Milan, Italy).

The decrease in light transmission determined by platelet shape change as described by Born [10] was continuously monitored by a pen recorder set at 0.1 volt full scale which gave a sensitivity of the instrument 10 times that used for platelet aggregation measurements. Maximal decrease (arbitrary units, a.u.) in light transmission was considered as a measure of platelet shape change [10,11].

Materials

All compounds were dissolved and diluted in isotonic saline; 5-hydroxy[G-^{3}H]tryptamine creatinine sulphate, specific activity 500 mCi/mmol, radiochemical purity 98%; [^{14}C]dopamine-HCl (3,4-dihydroxyphenyl[1-^{14}C]ethylamine-HCl), specific activity 59 mCi/mmol, radiochemical purity 99%, [^{14}C]tyramine (*p*-hydroxyphenyl[2-^{14}C]ethylamine-HCl), specific activity 50 mCi/mmol, radiochemical purity 98% were purchased from the Radiochemical Centre Amersham.

β-[*ethyl*-1-^{14}C]Phenethylamine-HCl, specific activity 48.25 mCi/mmol radiochemical purity 98.5% was purchased from New England Nuclear, Florence, Italy. Tyramine-HCl, serotonin, creatinine sulphate, dopamine-HCl, *β*-phenethylamine-HCl were obtained from Fluka AG, Buchs (Switzerland), methysergide hydrogen maleate from Sandoz Ltd., Basel, Switzerland and chlorimipramine-HCl from Ciba Geigy, Origgio, Italy.

Results

Comparison of the total uptakes of tyramine, dopamine, phenethylamine after 1 min incubation at 37°C is shown in Fig. 1. The results indicate that tyramine is taken up more efficiently than dopamine while phenethylamine is not taken up. The total uptake for tyramine and dopamine was concentration-dependent and yielded a two-phase curve which could be resolved into a rapid saturable phase (active transport: tyramine K_m 3.0 $\pm$ 0.028 μM, dopamine K_m 39 $\pm$ 5.9 μM) and a slower non-saturable phase, linear with concentrations (passive diffusion). In experiments conducted in parallel, the K_m for serotonin uptake was 0.35 $\pm$ 0.033 μM. The active uptake of tyramine and dopamine was competitively inhibited by serotonin ($K_i \sim 0.8$ μM), as was the uptake of [^{3}H]serotonin

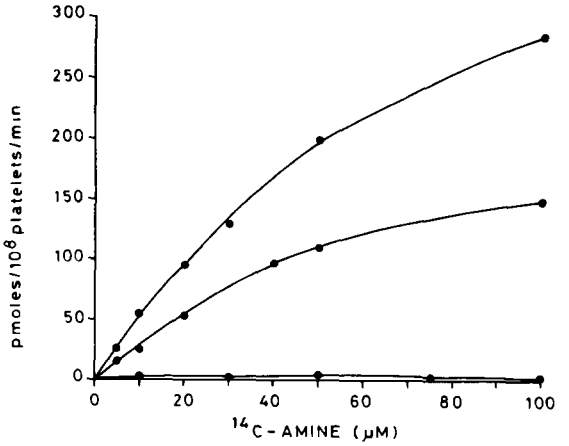

Fig. 1. Comparison of the total uptake of tyramine (top curve), dopamine (middle curve) and phenethylamine (bottom curve) by rat platelets after 1 min incubation at 37°C. The values shown were calculated by subtraction of the corresponding values obtained in blank samples incubated at 4°C as described [8,9].

TABLE I

THE APPARENT K_i VALUES (μM) OF THE INHIBITORY EFFECTS OF PHENETHYLAMINE DERIVATIVES, CHLORIMIPRAMINE AND METHYSERGIDE UPON SEROTONIN UPTAKE AND SEROTONIN-INDUCED SHAPE CHANGE

Means ± S.E.

	Serotonin uptake	Serotonin-induced shape change
Tyramine	12 ± 1.1	39 ± 4.5
Dopamine	145 ± 14.7	192 ± 8.7
Phenethylamine	112 ± 12.4	96 ± 15.6
Chlorimipramine	0.033 ± 0.004	0.631 ± 0.092
Methysergide	310 ± 25	0.017 ± 0.0027

by both amines (K_i ~ 12 and 145 μM for tyramine and dopamine, respectively, Table I). Despite the apparent lack of phenethylamine transport by rat platelets even after 30 min incubation at concentrations ranging from 1 to 500 μM, it competitively inhibited the uptake of [^{3}H]serotonin with a K_i of about 110 μM (Table I and Fig. 2).

The inhibitory effect of phenethylamine was not modified by incubating platelets with this amine from 0 to 30 min before adding the radioactive serotonin.

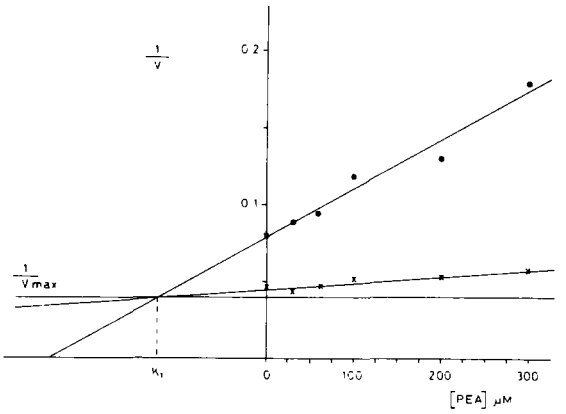

Fig. 2. Competitive inhibition of 0.3 μM (upper curve) and 3.3 μM (lower curve) [^{3}H]serotonin uptake by different concentrations of phenethylamine. The Dixon plot [12] was obtained by plotting the reciprocal of the [^{3}H]serotonin uptake ($1/$(pmol/10^8 platelets per 10 s), $1/V$) against the phenethylamine concentrations. The points were fitted by regression lines. The K_i of phenethylamine inhibition of [^{3}H]serotonin uptake in this particular experiment is 125 μM.

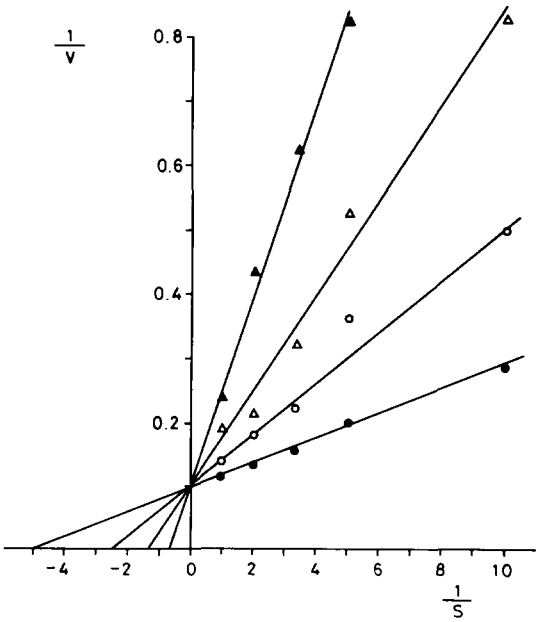

Fig. 3. Competitive inhibition of serotonin-induced shape change by tyramine. Serotonin concentrations ranged between 0.1 and 1.0 μM. Tyramine concentrations were the following: ○——○, 50 μM; △——△, 100 μM; ▲——▲, 300 μM; ●——●, control. The Lineweaver-Burk plot [13] was obtained by plotting the reciprocal of shape change arbitrary units ($1/V$) against the reciprocal of serotonin concentrations ($1/S$).

In view of the inhibitory effects of tyramine, dopamine and phenethylamine upon [^{3}H]serotonin uptake by platelets, we examined the effects of these compounds on serotonin-induced platelet shape change. From dose-response curve of platelet shape change induced by serotonin (0.1–10 μM), an apparent K_m of 0.31 ± 0.027 μM was calculated. Tyramine, dopamine and phenethylamine did not induce platelet shape change, although they all competitively inhibited serotonin-induced shape change with K_i values of 39, 192 and 96 μM, respectively (Table I). A typical experiment with tyramine is shown in Fig. 3. Table I also reports the K_i of the inhibitory effect of chlorimipramine and methysergide upon serotonin uptake and serotonin-induced shape change.

Discussion

The present study confirms [8,9] previous observations that tyramine and dopamine are taken

up by rat platelets through the serotonin uptake mechanism. Indeed, the saturable components of both tyramine and dopamine uptake were competitively inhibited by serotonin with a K_i (~ 0.8 μM) very closed to the apparent K_m of serotonin uptake (~ 0.3 μM). Conversely, both amines competitively inhibited [^{3}H]serotonin uptake with K_i values of the same order of magnitude as their corresponding apparent K_m values of uptake.

The apparent K_m values of dopamine uptake (~ 39 μM) and tyramine uptake (~ 3 μM) indicate that the monohydroxylated amine (tyramine) has a greater affinity for the transport system. On the other hand, the apparent failure of platelets to take up the non-hydroxylated derivative (phenethylamine) shows that an aromatic hydroxyl group is an absolute structural requirement for transport of phenethylamine derivatives through the serotonin uptake mechanism. Although phenethylamine is not taken up by platelets, it inhibits the uptake of serotonin by an apparent competitive mechanism (Table I and Fig. 2).

These observations suggest at least two separate steps in the serotonin uptake mechanism: receptor binding, accomplished to some degree by both phenethylamine and its derivatives, and translocation for which an aromatic hydroxyl group is a structural requirement and, consequently, not accomplished by phenethylamine. Although an hydroxyl group is essential for transport, the higher K_m for dopamine, as compared to tyramine transport, suggests that the presence of two hydroxyl groups significantly diminishes affinity to the receptor for serotonin uptake. The crucial role of the aromatic hydroxyl group in the uptake of serotonin and its structural analogues was already pointed out by Born et al. [3]. A number of investigators have shown that platelets possess serotonin receptors with different affinities. Drummond and Gordon [5] demonstrated the presence of three saturable binding sites on rat platelets while more recently Peters and Grahame-Smith [6] identified high (K_d 0.5–1 nM) and low (K_d 15–36 nM) affinity serotonin receptors on the surface of human platelets. Inhibitors of platelet shape change (such as methysergide) inhibit serotonin binding to the high affinity receptor while inhibitors of serotonin uptake (such as chlorimipramine) inhibit binding to the low affinity receptor [5,6]. This would seem to be in conflict with our observation that the K_m values for serotonin uptake and serotonin-induced shape change are virtually identical at ~ 0.3 μM. However, as has been suggested by Peters and Grahame-Smith [6], the inability to demonstrate a lower K_m for serotonin-induced shape change may reflect limited sensitivity of the optical system employed in its measurement.

As already proposed by Born [3,4] the lack of correlation between the potencies of chlorimipramine and methysergide (Table I) as shape change and uptake inhibitors is persuasive evidence that these two effects are mediated by different receptors. Our study supports this hypothesis in that tyramine and dopamine, both of which are taken up through the serotonin transport system, do not induce platelet shape change. Thus interaction with the serotonin transport mechanism is not sufficient to activate platelet shape change. On the other hand, phenethylamine derivatives inhibit serotonin transport and serotonin-induced shape change with the same relative inhibitor potencies (tyramine > phenethylamine $\geqslant$ dopamine). This suggests that the receptors controlling serotonin uptake and serotonin-induced shape change have a similar or common structural component that binds phenethylamine and its derivatives and permits them to act as inhibitors of both reactions. Tyramine and dopamine which also contain aromatic hydroxyl groups, are able to activate the serotonin transport mechanism, while phenethylamine with no aromatic hydroxyl groups is unable to activate either reaction. Thus, the study of the effects of phenethylamine derivatives upon rat platelet serotonin transport and shape change demonstrates a common binding requirement for both platelet reactions. However, the failure of the hydroxylated phenethylamine derivatives to induce both the uptake and the shape change is consistent with the concept that serotonin uptake and serotonin-induced platelet shape change are mediated by two distinct activation sites of the serotonin receptors.

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