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THE UPTAKE OF TYRAMINE BY RAT PLATELETS*

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Abstract—The uptake of [^{14}C]tyramine by rat platelets was less efficient than the uptake of [^{14}C]-5-HT. [^{14}C]-Tyramine was taken up in a two phase curve which could be resolved into a rapid saturable phase and a slower non-saturable phase that was linear with concentration. The value of the non-saturable component was ~ 12 pmoles/10^8 platelets/15 sec at 10 μM tyramine concentration. The K_m of active transport was ~ 3 μM and the V_{max} was ~ 25 pmoles/10^8 platelets/15 sec. [^{14}C]Tyramine active uptake was inhibited by both serotonin (K_i ~ 0.8 μM) and fenfluramine (K_i ~ 2.0 μM). These results strongly suggest that the active uptake of tyramine by rat platelets is accomplished by means of the 5-HT transport mechanism. The affinity of tyramine for the 5-HT receptor (~ 3 μM) is substantially higher than the affinity reported for dopamine (~ 70 μM).

This indicates that the 3-hydroxyl group of dopamine diminishes its affinity for the 5-HT receptor of rat platelets.

The observation that human blood platelets take up dopamine by an energy dependent, saturable process [1, 2] suggested that platelets might be an appropriate model for the transport of dopamine in the central nervous system. However, recent studies have failed to reveal specific platelet binding of dopamine [3] and have demonstrated that its uptake by platelets is accomplished through the serotonin uptake mechanism [4, 5]. These observations suggest that the uptake of dopamine by platelets may be merely the result of structural similarities between serotonin (5-hydroxytryptamine, 5-HT) and β-phenylethylamine derivatives [6]. In order to test this hypothesis, we examined the uptake of tyramine by platelets.

MATERIALS AND METHODS

Rat blood was collected in a plastic syringe from 250–300 g CD-COBS rats (Charles River; Calco, Italy) by intracardiac puncture following anesthetization with ether. The blood was placed in 0.126 M trisodium citrate in a 9:1 volume mixture. Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared by differential centrifugation as described previously [7]. For each experiment, a pool of PRP from at least three animals was used. The platelet count was adjusted to 600,000/μl by appropriate dilution with autologous PPP and all studies were performed within 1–3 hr after blood collection.

Uptake of [^{14}C]*tyramine and* [^{14}C]*-5- hydroxytryptamine (*[^{14}C]*-5-HT).* The uptake of [^{14}C]tyramine and [^{14}C]-5-HT was measured according to the method of Gordon and Olverman [4]. In brief, 0.2 ml samples of PRP were preincubated at 37° for 0–5 min before addition of the [^{14}C]-amine. The uptake reactions were stopped at various intervals by the addition of cold disodium-EDTA–saline. The samples were then immediately centrifuged in an Eppendorf microcentrifuge (60 sec; 10,000 *g*). The platelet pellets were digested in 0.2 ml of 26 M formic acid for one hour before transfer to plastic mini-vials containing 5 ml of the scintillant described by Gordon and Olverman [4]. Radioactivity was measured in a liquid scintillation counter. Replicate samples in which the [^{14}C]-amine was added after the PRP was cooled to 4° provided a measure of the radioactivity trapped within the cell pellets. The uptake of [^{14}C]-amine was calculated by subtracting the amount of radioactivity that was trapped from the total radioactivity obtained in the incubated sample. Substrate depletion did not exceed three per cent in any of the experiments.

Materials. All compounds were dissolved in isotonic saline. [^{14}C]Tyramine (*p*-hydroxyphenyl [2-^{14}C]ethylamine hydrochloride, specific activity 50 mCi/mmole, radiochemical purity 97%) and [^{14}C]-5-hydroxytryptamine (5-hydroxy-3-indolyl-[^{14}C]-ethyl-2-amine creatinine sulfate monohydrate, specific activity 58 mCi/mmole, radiochemical purity 97%) were purchased from Amersham, Prodotti Gianni, Italy. The following non-radioactive compounds were obtained from the indicated sources: tyramine hydrochloride (Sigma); 5-HT creatinine sulfate (Fluka AG, Buch, Switzerland); *d*-fenfluramine (Servier, Paris, France).

RESULTS

The uptake of [^{14}C]tyramine by rat platelets was concentration dependent but less efficient than the

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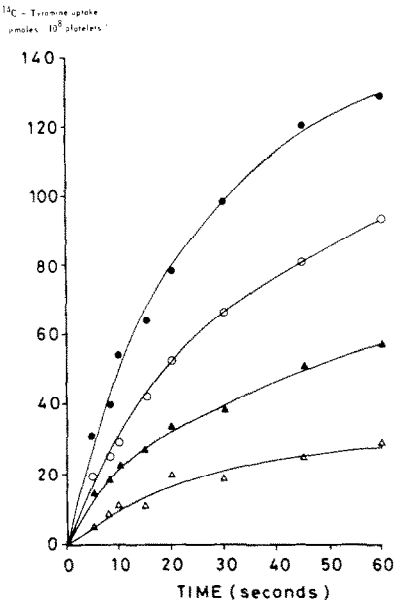

Fig. 1. The uptake of [^{14}C]tyramine by rat platelets. The concentrations of [^{14}C]tyramine used were 5 μM (△), 10 μM (▲), 20 μM (○), and 30 μM (●).

uptake of [^{14}C]-5-HT. In the presence of 30 μM [^{14}C]tyramine, rat platelets took up 125 pmoles/10^8 platelets/min (Fig. 1). By comparison, parallel incubations with 2 μM [^{14}C]-5-HT yielded an uptake of 150 pmoles/10^8 platelets/min (data not shown). Thus the uptake of 5-HT by platelets was approximately twenty times more efficient than the uptake of tyramine.

The uptake of [^{14}C]tyramine was linear for only a brief period of time. In order to determine the kinetics of tyramine uptake, experiments were repeated using an incubation period of 15 sec. The

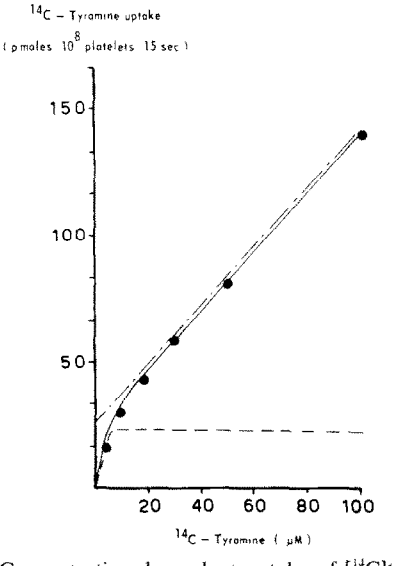

Fig. 2. Concentration-dependent uptake of [^{14}C]tyramine by rat platelets. The linear portion of the experimental curve (———) was extrapolated to the ordinate (– . –) and utilized to calculate the active uptake (– – –).

results, plotted against tyramine concentration, yielded a two phase curve which could be resolved into a rapid saturable phase and a slower non-saturable phase that was linear with concentration (Fig. 2). The value of the non-saturable uptake was ~ 12 pmoles/10^8 platelets/15 sec at 10 μM tyramine concentration. Extrapolation of the slower linear function intercepted the ordinate at 25 pmoles/10^8 platelets. This intercept represented the theoretical maximum (V_{max}) of the saturable phase of tyramine uptake at 15 sec and had an apparent K_m of 2–3 μM.

In order to confirm these kinetic values for initial saturable uptake, incubation studies were performed using a range of [^{14}C]tyramine concentrations (0.5–5 μM) which were closer to the apparent K_m. The resulting data confirmed that the rate of uptake was more rapid during the first 15 sec and indicated that saturation of this initial phase appeared to occur at ~ 2.5 μM (Fig. 3). When uptake was plotted against concentration and transformed to a linear function by Woolf analysis [18], the reciprocals obtained with 15, 30 and 60 sec incubation times converged to the same intercept on the abscissa indicating a K_m of ~ 3 μM (Fig. 4). The V_{max} at 15 sec was ~ 30 pmoles/10^8 platelets and also agreed closely with the theoretical value. Further confirmation of these kinetic values was obtained by subjecting the 15 sec uptake data shown in Fig. 3 to a Lineweaver–Burk plot [8]. This form of analysis yielded a K_m of 2.8 μM and a V_{max} of 24 pmoles/10^8 platelets (Fig. 5).

The structural similarities of tyramine and 5-HT suggest that active uptake of both amines could occur through the same receptor. In order to test this hypothesis, the effect of non-radioactive 5-HT upon the uptake of [^{14}C]tyramine was investigated. In the presence of 2 μM 5-HT, the uptake of [^{14}C]tyramine became a single phase linear process which yielded a line parallel to the second phase of tyramine uptake obtained when 5-HT was withheld. These data indicate that 5-HT is an inhibitor of active tyramine uptake with no effect upon the passive diffusion of tyramine. When the uptake of [^{14}C]tyramine in the presence of varying 5-HT concentrations was subjected to a Dixon plot [9], 5-HT was revealed to be an apparent competitive inhibitor of tyramine uptake with a K_i of ~ 0.8 μM (Fig. 6). This conclusion was further confirmed in tyramine uptake studies performed in the presence of *d*-fenfluramine a competitive inhibitor of 5-HT uptake [18]. These studies indicated that *d*-fenfluramine was also an apparent competitive inhibitor of tyramine uptake with a K_i of ~ 2 μM.

In other experiments, samples of PRP were preincubated with 5-HT (2 μM) for 0–4 min before addition of [^{14}C]tyramine (20 μM). The inhibition of tyramine uptake by 5-HT was maximal immediately (0 time, 71% inhibition) and diminished progressively during preincubation (1 min, 50%; 2 min, 40%; 4 min, 35%) suggesting that the inhibitory effect of 5-HT on tyramine uptake is exerted at the level of the plasma membrane.

DISCUSSION

Serotonin is taken up by platelets through a process of active transport and passive diffusion [10–

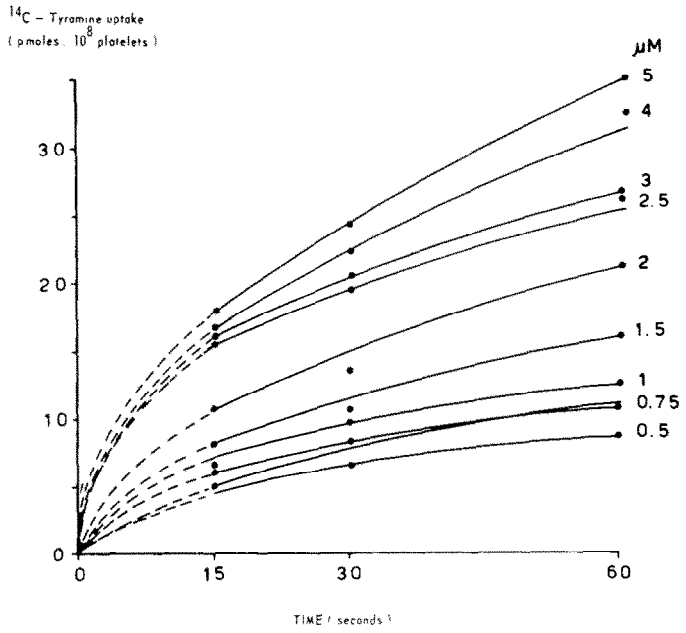

Fig. 3. The uptake of [^{14}C]tyramine by rat platelets utilizing [^{14}C]tyramine concentrations (0.5–5 μM) which are within the range of the K_m for active transport.

12]. These observations suggested that the study of 5-HT transport by platelets might provide information applicable to serotonergic neurons [13]. This conclusion was reinforced by Tuomisto [14] who showed that studies performed under the appropriate conditions revealed remarkable similarities between the kinetics of 5-HT transport by rabbit platelets and by rat synaptosomes. It was further supported by Drummond and Gordon [15, 16] who demonstrated specific 5-HT binding sites on the platelet membrane. However, the uptake of dopamine by platelets has not yielded conclusions applicable to dopaminergic neurons.

Although dopamine uptake by platelets can be resolved into saturable and non-saturable components [4, 5], Boullin *et al.* [3] were unable to detect specific dopamine binding sites on human platelets. Moreover, in rats the affinity of dopamine for the platelet ($K_m \sim 70\,\mu$M) [4], is much less than its affinity for the dopaminergic neuron ($K_m \sim 0.13\,\mu$M)

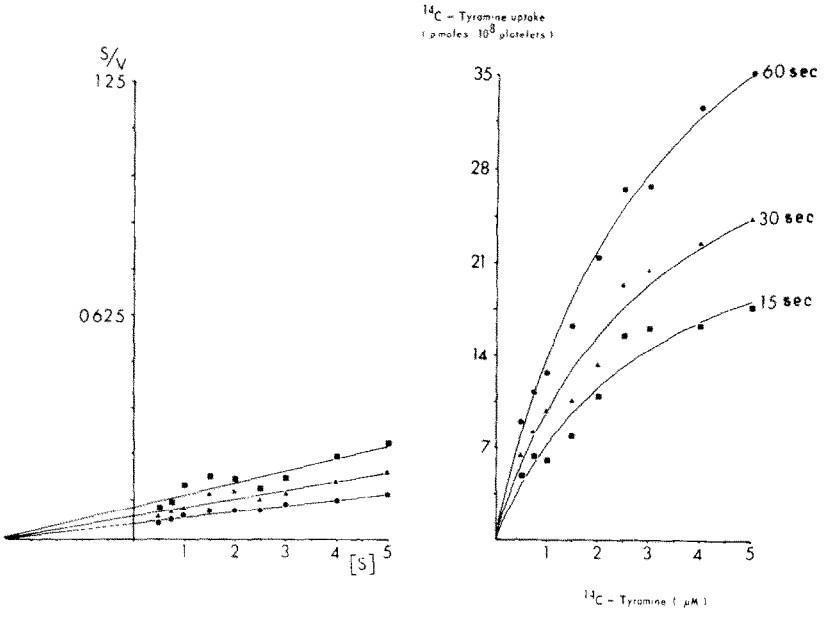

Fig. 4. Woolf analysis of the data reported in Fig. 3. The hyperbolic curves (right panel) were subjected to the linear transformation, S/V versus V (left panel) and an apparent K_m value of 3 μM was obtained (by an appropriate computer program).

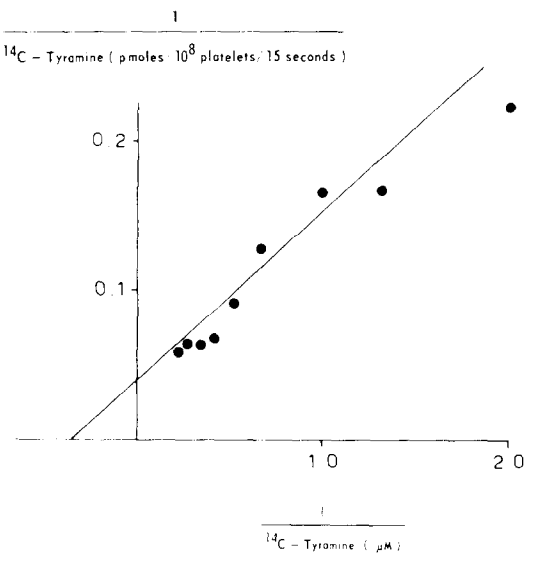

Fig. 5. Lineweaver–Burk plot of the 15sec [^{14}C] tyramine uptake data shown in Fig. 3.

[17]. Finally, Gordon and Olverman [4] and Omenn and Smith [5] have convincingly demonstrated that the uptake of dopamine by human and rat platelets is an inefficient process accomplished through the serotonin uptake mechanism. Our studies indicate that the platelet handles [^{14}C]tyramine in a similar manner.

The uptake of [^{14}C]tyramine by rat platelets is a biphasic curve that can be resolved into saturable and non-saturable components. The K_m of the saturable component is ~ 3 μM and it is competitively inhibited by 5-HT with a K_i (0.8 μM) which is well within the range of the K_m of 5-HT uptake by rat platelets (0.7–1.2 μM) [4, 18]. The K_i of the inhibitory effect of *d*-fenfluramine upon tyramine uptake (2.0 μM) is also close to the K_i of its inhibitory effect upon serotonin uptake (1.3 μM) [18]. Preincubation of 5-HT with platelets diminishes the inhibitory effect of 5-HT upon tyramine uptake and, as shown by Gordon and Olverman [4], upon dopamine uptake as well. These findings suggest that tyramine and dopamine are both taken up by the serotonin active transport site at the platelet plasma membrane. However, the affinity of tyramine for the 5-HT receptor of the rat platelet (K_m ~ 3 μM) is substantially greater than the affinity demonstrated by dopamine (K_m ~ 70 μM) [4]. This difference must be due to the 3-hydroxylation of the phenyl ring in dopamine, and presumably, to the effect this substitution must have on the binding of β-phenylethylamine derivatives to the 5-HT receptor of platelets [6].

Born *et al.* [19] have demonstrated that the 5-hydroxyl group is critical to the uptake of the 5-HT by platelets while Marcus *et al.* [20] have shown that it is also required for the binding of hydroxyindole analogues by ganglioside extracts of platelet membranes. On the other hand, 5,6-dihydroxytryptamine (5,6-DHT) is a competitive inhibitor of 5-HT uptake with a K_i of ~ 46 μM [21]. This indicates that the additional hydroxyl group at the six position diminishes the affinity for the 5-HT receptor. Beta phenylethylamine (PEA) derivatives have similar structure activity relationships to the 5-HT receptor. Richter and Smith [6] examined the inhibitory effect of forty eight PEA derivatives upon the uptake of 5-HT by human platelets. Their results indicated that 3-hydroxylation of the ring decreased inhibitory potency (*d*-amphetamine > 3-hydroxyamphetamine) while 4-hydroxylation led to augmentation (tyramine > PEA). In their studies dopamine was less effective than tyramine so that the rank order of inhibitors of 5-HT uptake indicated that tyramine > dopamine > PEA.

Our observation that tyramine has a greater affinity for the 5-HT receptor than dopamine [4] is consistent with the studies of Richter and Smith [6]. It is also consistent with the early observations of

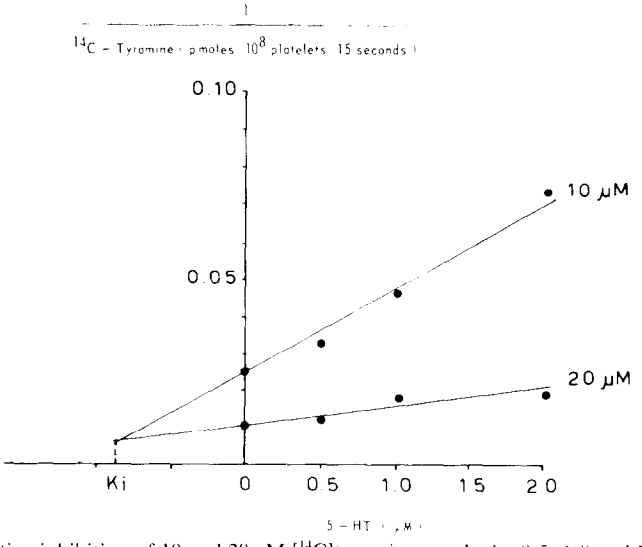

Fig. 6. Competitive inhibition of 10 and 20 μM [^{14}C]tyramine uptake by 0.5, 1.0 and 2.0 μM 5-HT. The Dixon plot was obtained by plotting the reciprocal of the [^{14}C]tyramine uptake ($1/V$) against the 5-HT concentration. The K_i of the serotonin inhibition of [^{14}C]tyramine uptake is ~ 0.8 μM.

Stacey [22] which also showed that tyramine was more potent than dopamine as an inhibitor of 5-HT uptake by platelets. These studies strongly suggest that the uptake of β-phenethylamine derivatives by platelets is a reflection of structural homologies that permit the uptake of these compounds through a platelet receptor transport system specific for 5-HT. On the other hand, Baldessarini and Vogt [23] demonstrated that the K_m of tyramine uptake by rat brain homogenates was 3.2 μM. That value is surprisingly close to the K_m of tyramine uptake by rat platelets. Whether this similarity is fortuitous or indicative of some specific analogous function is open to conjecture. However, in this connection the differences in 5-HT uptake by mouse platelets and mouse synaptosomes recently described by Smith *et al.* [24] suggest that there are limits to the ability of platelets to mirror neuronal function.

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