Effect of Monase and Related Compounds on Uptake of 5-Hydroxytryptamine by Platelets.* (31233)

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5-Hydroxytryptamine (5-HT) is taken up actively by platelets against a concentration gradient of several hundred to one and its uptake is energy- and temperature-dependent (1,2). An active transport system at the platelet surface has been suggested(3). Tryptamine in low concentrations inhibits the uptake of 5-HT by platelets presumably by competing for the same transport site(1). However, although differing from 5-HT only by the absence of a hydroxyl group, tryptamine is concentrated by platelets to a level only 2-3-fold that of the surrounding medium (1). This indicates a high degree of structural specificity for the transport site.

In the present study the structural specificity for 5-HT transport has been investigated further by examining the effect of the monoamine oxidase inhibitor Monase indole, 3-(2-aminobutyl) acetate, and 8 other structurally related compounds with different substitutions on the indole ring and amine side chain.

Materials and methods. 5-Hydroxytryptamine creatinine sulfate labeled with C^{14} at the β-carbon atom and with a specific activity of 6.59 μc/mg was obtained from the New England Nuclear Co., Boston. C^{14}-labeled Monase, specific activity 2.8 μc/mg, unlabeled Monase and eight other structurally related compounds (U15,655; U13,783E; U14,-164E; U22,259A; U16,967E; U14,077E; U11,300A and U18,152A) (Fig. 1) were obtained through the courtesy of Dr. Walter Anglin, Upjohn Co., Kalamazoo. Asparagine, tryptophan, lysine, phenylalanine, and alanine were obtained from Mann Research Laboratories, New York. Epinephrine and norepinephrine were purchased from commercial sources.

All glassware with which platelets came in contact was siliconized. Blood was collected from normal human volunteers by direct venipuncture into 50 ml centrifuge tubes containing EDTA (final concentration 0.005 M). The blood was rapidly cooled in ice and platelet-rich plasma was prepared by centrifugation at 120 $\times$ g for 20 minutes at 4°C. Three milliliter aliquots of platelet-rich plasma were transferred to 25 ml incubation flasks. Each test compound was added in the indicated final concentration in a volume of 0.1 ml. The control flasks received 0.1 ml of saline. After a preincubation period of 15 minutes at 37°C, C^{14}-labeled 5-HT was added to each flask to give a final concentration of 2.57×10^{-5} M and incubation continued for an additional 30 minutes in a Dubnoff metabolic shaker. After incubation the platelet suspensions were transferred to pre-cooled McKnaught tubes and centrifuged at 1800 $\times$ g for 30 minutes. The pH of the supernates ranged between 8.1 and 8.4. The cells were washed twice with 6 ml of buffered saline, then lysed in 1 ml of distilled water by rapid mixing with a glass stirring rod. Samples of 0.1 ml of suspension were plated on stainless steel planchettes and their radioactivity assayed in a gas flow counter. Results were expressed as CPM/ml of lysed platelet suspension at infinite thinness.

Results. The results of a representative experiment testing the effect of 3 structurally related compounds on the course of uptake of 5-HT by platelets are shown in Fig. 2. 5-HT uptake was maximal at 30 minutes as was the inhibition of uptake by all 3 inhibitors.

The effect of increasing concentrations of inhibitors on 5-HT uptake is shown in Table I. The degree of inhibition increased with higher concentrations of all compounds amounting to approximately 65% at concen-

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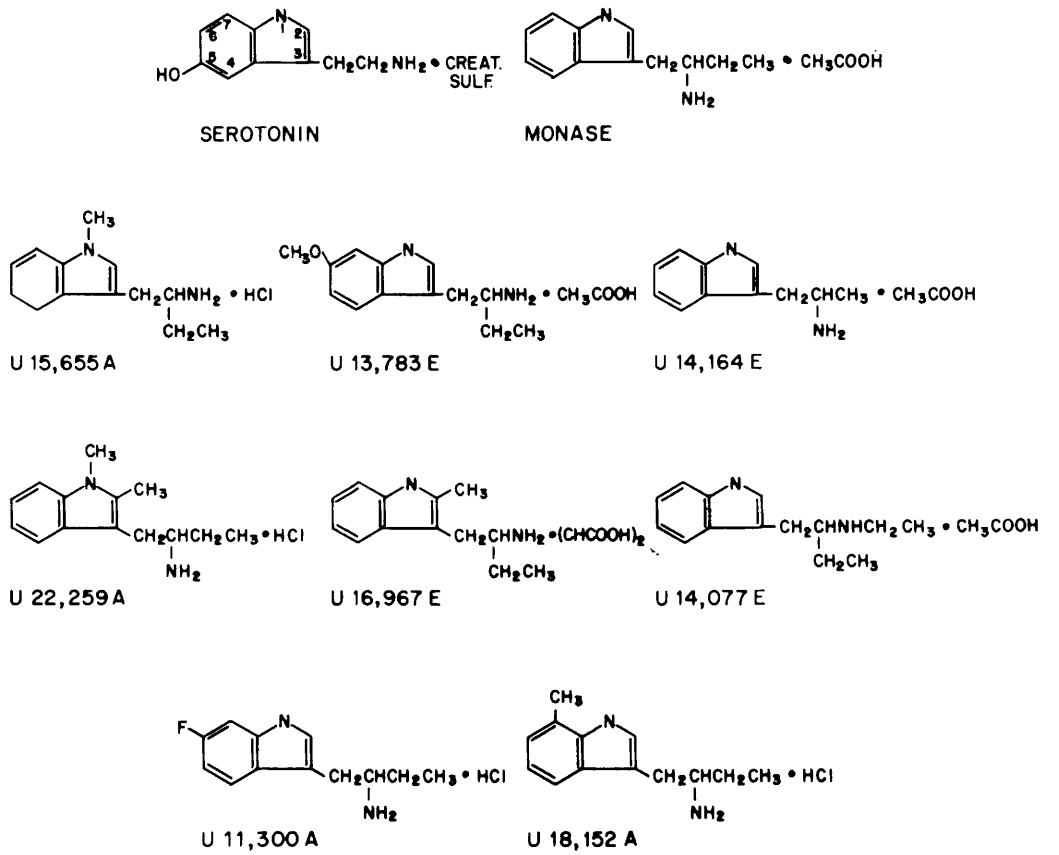

FIG. 1. Structure of 5-hydroxytryptamine, Monase and 8 other related compounds.

trations 10 times that of C^{14} 5-HT. However, significant inhibition occurred when the concentration of inhibitor was only twice that of C^{14} 5-HT. This appeared particularly true with U14,077E and U18,152A. Little difference in the inhibitory capacity of the 8 compounds was noted at high concentrations. Furthermore the degree of inhibition was identical with one or a combination of two or more compounds provided the total molar concentration remained the same (not illustrated). This indicated a direct relation of the degree of inhibition to the concentration of inhibitors and little or no relation to their structural differences.

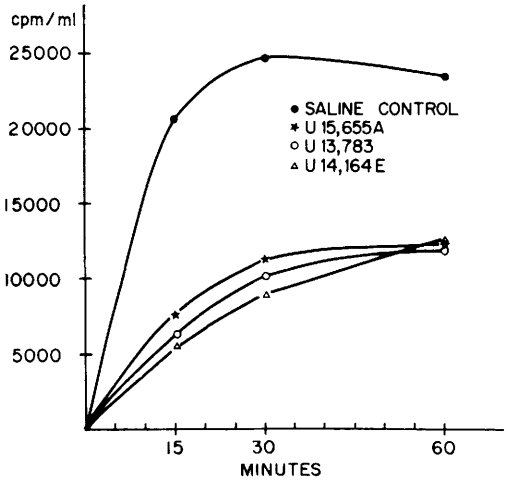

FIG. 2. Effect of 3 structurally related compounds on the course of uptake of 5-hydroxytryptamine by platelets.

C^{14}-Monase was taken up by platelets to a concentration 1/25th that of 5-HT (Table II). However, the uptake of both 5-HT and Monase was markedly enhanced by K^+, and stimulated by glucose and PO_4^{3-}. The combination of K^+ and glucose was not additive.

The effect of other amines and some amino acids was also tested. Significant inhibition

TABLE I. Pattern of Inhibition of Uptake of C^{14} 5-HT by Monase and Other Analogs at Various Concentrations. C^{14} 5-hydroxytryptamine concentration, 2.57×10^{-5} M.

$$\% \text{ Uptake} = \frac{\text{CPM (analog)}}{\text{CPM (saline control)}} \times 100$$

Concentration of analog	Saline control	Monase	U15,655A	U13,783E	U14,164E	U22,259A	U16,967E	U14,077E	U11,300A	U18,152A
4.96×10^{-5} M	100	75	77	77	68	72	77	60	65	58
1.48×10^{-4} M	100	46	46	47	43	56	60	44	52	48
2.45×10^{-4} M	100	37	33	36	32	45	45	37	38	43

TABLE II. Comparative Uptake of 5-HT and Monase by Platelets.

	Concentration (mμmoles/ml)	
	Medium*	Packed platelets
5-HT-C^{14}	5.0	600
Monase-C^{14}	5.0	29

* Values represent concentrations prior to incubation.

of 5-HT uptake occurred only with epinephrine and norepinephrine, there being no effect from asparagine, lysine, alanine, phenylalanine or tryptophan.

Discussion. The uptake of 5-HT by platelets is carried out by an active transport process and is markedly influenced by pH and temperature changes. The uptake is enhanced by K^+ and PO_4^{3-} ions and is inhibited by fluoride, 2,4-dinitrophenol, and cardiac glycosides known to inhibit cationic transport(4). Chlorpromazine, imipramine, reserpine and tyramine also inhibit uptake (1).

It has been shown that platelets have an unusually high concentration of adenosine triphosphate and that the ATP content of platelets and their 5-HT uptake are directly related(2). The suggestion has also been made that the cationic amine is linked to the negatively charged nucleotide. This hypothesis, however, fails to explain why other amines are not taken up by platelets as actively as 5-HT. While some amines such as epinephrine inhibit the uptake of 5-HT and are themselves taken up by platelets perhaps by similar mechanisms, their uptake is very small compared to that of 5-HT. This high affinity of the transport site for 5-HT is not readily explainable. In the present study, 7 of the inhibitors tested are primary and 2 are secondary amines, with varying length of the amine chain. Inhibition of 5-HT uptake required relatively high concentrations of each inhibitor and was of approximately the same degree for all the compounds, indicating that the nature and length of the amine side chain is probably not a major factor in uptake. Other substitutions on the indole ring also seemed without effect. Monase is very similar in structure to 5-HT yet is taken up by platelets to a much lesser extent. These find-

ings and those of Stacey(3) showing that tryptamine is relatively little concentrated by platelets suggest strongly that the structural specificity is largely determined by the hydroxyl group at position 5 of the indole ring.

Summary. Monase indole-3(2-aminobutyl) acetate, a monoamine oxidase inhibitor, and 8 other structurally related compounds with different substitutions on the indole ring and amine side chain inhibited 5-HT uptake by platelets, degree of inhibition being directly related to concentration of inhibitor without particular relation to structure. C^{14}-Monase which differs from 5-HT by the absence of a hydroxyl group in position 5 of the indole ring and by the presence of a different side chain, was concentrated only slightly by platelets. It was concluded that the structural specificity for 5-HT uptake is largely determined by the hydroxyl group at position 5 of the indole ring.

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Hemagglutinins for DNA in Tuberculosis and Histoplasmosis.* (31234)

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Previous reports(1) of auto-tissue substances in tuberculosis have indicated that the basic disease process may be complicated by a secondary immunological response to unknown tissue antigens. This investigation shows that sera from rabbits experimentally infected with tuberculosis and sera from humans with tuberculosis and histoplasmosis contain antibodies, demonstrable by hemagglutination, to a known auto-antigen, DNA.

Sera from 14 of 16 rabbits experimentally infected with bovine tubercle bacilli demonstrated antibodies at some stage of infection. Similar tests were performed on the sera from humans with either tuberculosis or histoplasmosis.

Materials and methods. Serology. The bisdiazotized benzidine (BDB) indirect hemagglutination test described by Bigley *et al*(2), was used. The sensitivity of this test in detection of anti-DNA in SLE sera was found equally as effective as the bentonite method of Bozicevich(3).

Phosphate buffer (pH 7.3) was used throughout as both solvent and diluent. BDB was prepared according to the method of Gordon *et al*(4). The antigen was prepared by coupling thermally denatured DNA to human group O Rh positive erythrocytes with BDB. DNA (Nutritional Biochemicals Corp.) was denatured by boiling a solution for 10 minutes in a water bath, followed by immediate cooling in an ice bath(5).

One-tenth ml of washed, packed red cells was added to 10.0 ml of phosphate buffer containing 1000 μg of denatured DNA; 0.5 ml of 1:16 BDB was added and the reaction was allowed to proceed for 10 minutes at room temperature accompanied by frequent shaking of the mixture. After centrifugation, the supernate was discarded, and the treated erythrocytes were washed with 1:100 serum-phosphate buffer solution (1.0 ml normal serum + 99.0 ml buffer), and the sensitized red cells were prepared as a 2% suspension in the serum buffer.

Serum samples in 0.7 ml amounts were diluted 1:2, inactivated at 56°C for 30 minutes, and absorbed with 0.4 ml of washed, packed, normal human group O Rh positive red cells for 30 minutes at 37°C. Serial dilu-

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