Biochemical Pharmacology, 1965, Vol. 14, pp. 1135–1139. Pergamon Press Ltd., Printed in Great Britain.

ALTERATIONS OF THE 5-HYDROXYTRYPTAMINE OUTFLUX FROM BLOOD PLATELETS *IN VITRO*

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(*Received* 22 *February* 1965; *accepted* 11 *March* 1965)

Abstract—Glucose, fructose, ATP, but not saccharose, ribose, rhamnose, and pyruvate, counteract the rapid 5HT diminution of platelets incubated aerobically in glucose-free isotonic K-phosphate, pH 7·5. Reserpine, Ro 4-1284, iproniazid, and pargyline, but not DL-amphetamine, Ro 4-6861, tyramine, and guanethidine, also attenuate the 5HT decrease of platelets in the above buffer. It is concluded that glucose, fructose, and ATP partly restore the storing capacity of the platelets for 5HT, whereas reserpine, Ro 4-1284, iproniazid and pargyline interfere with the outflux of the 5HT through platelet membranes.

BLOOD platelets of rabbits preincubated in glucose-free isotonic K-phosphate, pH 7·5, and resuspended in fresh buffer rapidly lose their 5-hydroxytryptamine (5HT). Thereby, at best traces of 5HT metabolites are formed, whereas in glucose-containing media (Tyrode solution, plasma, K-phosphate + glucose) part of the released 5HT is transformed into 5-hydroxytryptophol and to a minor extent into 5-hydroxyindoleacetic acid. These findings led to the assumption that lack of energy supply might abolish active processes like 5HT storage. Reserpine counteracts the 5HT decrease of platelets suspended in glucose-free K-phosphate. This has been attributed to an interference of the drug with the outflux of the 5HT through the platelet membrane.[1]

The present paper deals with the effect of temperature as well as of various drugs, sugars, and metabolic intermediates on the outflux of 5HT from isolated platelets in glucose-free K-phosphate.

METHODS

Thrombocytes of rabbits were isolated as previously described and preincubated aerobically at 37° for 60 min in isotonic glucose-free K-phosphate, pH 7·5.[2] After centrifugation, the platelets were resuspended in fresh buffer of the same composition and incubated at 37° with or without drugs, sugars, and metabolic intermediates. Part of the resuspended platelets were incubated at 4° in glucose-free K-phosphate. The 5HT of the platelets was measured by a spectrophotofluorimetric method.[3]

RESULTS

1. The rapid decrease of the platelet 5HT, which occurs in glucose-free K-phosphate at 37°,[1] no longer takes place at 4°. Thus, within 1 hr of reincubation of the platelets at 37°, the 5HT decreases to 27 ± 3% as compared to the 5HT content of platelets immediately after resuspension. Platelets reincubated at 4° still contain 96 ± 4% 5HT after 1 hr (means of 8 experiments each ± SE).

2. As previously described, glucose attenuates the spontaneous depletion of platelet 5HT.[1] Concentrations as low as 0·1 mg/ml glucose have already a significant action; with higher amounts the effect becomes more marked. Among various other sugars, only fructose has a similar, though somewhat weaker effect, whereas saccharose, ribose, and rhamnose are without influence. Furthermore, 5′(pyro)-triphosphate of adenosine (ATP) but not pyruvate moderately attenuates the 5HT decrease in platelets (Fig. 1, Table 1).

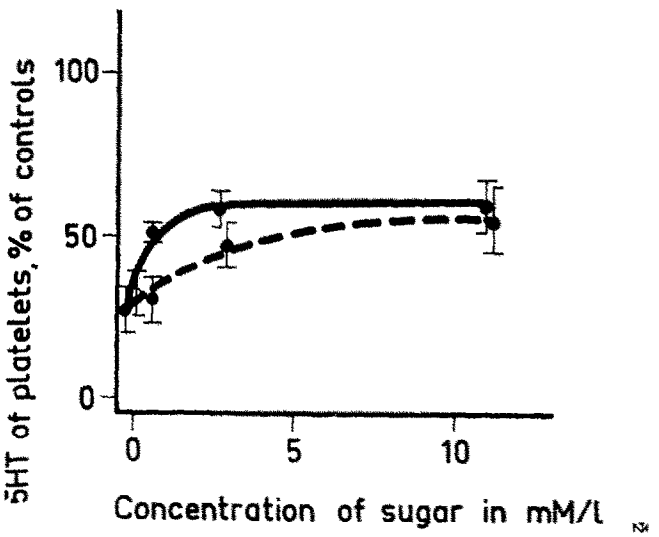

FIG. 1. Inhibition of the 5-hydroxytryptamine decrease of platelets in K-phosphate buffer at 37° by various concentrations of glucose or fructose.
The platelets were preincubated in K-phosphate for 1 hr, resuspended in the same buffer, and incubated for 2 hr. Controls = platelets immediately after resuspension; —— glucose; - - - - fructose. Each point represents an average of 3 experiments ± S.E.

TABLE 1. INFLUENCE OF VARIOUS SUGARS AND OF SOME PHYSIOLOGICAL INTERMEDIATES ON THE 5-HYDROXYTRYPTAMINE CONTENT OF ISOLATED BLOOD PLATELETS OF RABBITS

Substance added	5-Hydroxytryptamine	*P* compared to controls
Controls	20 ± 6	
Glucose	61 ± 6	<0·01
Fructose	51 ± 2	<0·01
Saccharose	23 ± 6	>0·05
Ribose	19 ± 5	>0·05
Rhamnose	28 ± 5	>0·05
Pyruvate	26 ± 5	>0·05
ATP	39 ± 3	<0·01

The platelets were preincubated at 37° for 60 min in K-phosphate buffer, pH 7·5, resuspended in the same buffer containing the sugars or intermediates, and incubated for 90 min at 37°. The sugars and pyruvate were added in amounts equimolar to 2 mg/ml glucose. Concentration of ATP: 2 mg/ml. The 5-hydroxytryptamine is indicated in percent of the platelet values immediately after resuspension. Each figure represents an average of 3 experiments ± S.E.

TABLE 2. EFFECT OF VARIOUS DRUGS ON THE 5-HYDROXYTRYPTAMINE CONTENT OF ISOLATED BLOOD PLATELETS OF RABBITS

Drugs	Concentration of drugs γ/ml	5-Hydroxytryptamine 60 min	5-Hydroxytryptamine 90 min
Controls	—	19 ± 2	12 ± 1
Reserpine	10	52 ± 4*	36 ± 5·5*
Ro 4-1284	5	38 ± 4*	24 ± 4*
	50	36 ± 3*	22 ± 4·5
DL-Amphetamine	13	19 ± 2	14 ± 2
(hydrochloride)	130	22 ± 2	14 ± 2
Ro 4-6861	20	23 ± 4	14 ± 4
(hydrobromide)	200	14 ± 2	14 ± 4
Tyramine	13	19 ± 3	11 ± 1
(hydrochloride)	130	18 ± 3	13 ± 2

The platelets were preincubated at 37° for 60 min in K-phosphate buffer, pH 7·5, resuspended in the same buffer, and incubated for 60 and 90 min at 37°. The drugs were added after resuspension of the platelets. The 5-hydroxytryptamine is indicated in percent of the platelet values immediately after resuspension. Each figure represents an average of 3 experiments ± S.E. The sympathomimetic amines were applied in equimolar amounts.

* $P < 0{\cdot}01$ (compared to controls).

TABLE 3. EFFECT OF MONOAMINE OXIDASE INHIBITORS AND GUANETHIDINE ON THE 5-HYDROXYTRYPTAMINE CONTENT OF ISOLATED BLOOD PLATELETS OF RABBITS

Drugs	Concentration of drugs (γ/cc)	5-Hydroxytryptamine 60 min	5-Hydroxytryptamine 120 min
Iproniazid	20·5	120 ± 7*	126 ± 9*
(phosphate)	205·0	142 ± 9†	146 ± 6†
Pargyline	14·5	135 ± 5†	164 ± 17†
(hydrochloride)	145·0	150 ± 9†	175 ± 7†
Guanethidine	18·3	99 ± 5	102 ± 4
(sulfate)	183·0	108 ± 4	103 ± 5

The platelets were preincubated at 37° for 60 min in K-phosphate buffer, pH 7·5, resuspended in the same buffer, and reincubated for 60 and 120 min at 37°. The drugs were added immediately after resuspension of the platelets. The 5-hydroxytryptamine is indicated in percent of the 5-hydroxytryptamine values of platelets reincubated for 60 and 120 min respectively without addition of drugs (=100%). The drugs were applied in equimolar amounts.

* $0{\cdot}01 > P < 0{\cdot}05$ compared to controls.

† $P < 0{\cdot}01$ compared to controls.

3. The benzoquinolizine derivative Ro 4-1284*,[4] similarly to reserpine, counteracts the decrease of platelet 5HT, whereas DL-amphetamine, Ro 4-6861,† tyramine, and guanethidine are without effect (Table 2). The monoamine oxidase inhibitors iproniazid and pargyline also attenuate the depletion of platelet 5HT (Table 3).

* 2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo [a] quinolizine.

† DL-4-chloro-N-methylamphetamine[5].

DISCUSSION

The rapid liberation of 5HT from platelets incubated in glucose-free K-phosphate is likely to be due to an abolition of the active 5HT storage which probably depends on energy supply.[6] Therefore, in a glucose-free medium the condition of the platelet membrane becomes probably a main factor which determines the rate of outflux of 5HT from the platelets. Based upon the present findings, it cannot be decided whether the passage of 5HT through the platelet membrane is an active process. The fact that at low temperature the decrease of the platelet 5HT is abolished might support such an assumption. On the other hand, it is unlikely that an active transport mechanism persists if active storage and metabolic processes are abolished.

The present results indicate that fructose and ATP act similarly to glucose, and it is conceivable that these three substances are energy supplying substrates which partly restore the storage mechanism of the platelets for 5HT. Thereby, fructose is somewhat less effective than glucose indicating metabolic differences of the two sugars. Saccharose, ribose, and rhamnose do not affect the 5HT decrease, probably because these sugars are not metabolized by the platelets *in vitro*. Pyruvate, which does not influence the 5HT increase either, might be unable to enter the platelets in sufficient amounts under the present experimental conditions.

The above results show furthermore that monoamine-liberating drugs do not enhance the 5HT decrease of the platelets in K-phosphate buffer. These agents seem to cause monoamine liberation only in the presence of an intact storage mechanism (e.g. in plasma or Tyrode solution)[7–9] which is abolished in the absence of glucose. Two types of monoamine liberators can be distinguished by their action in K-phosphate buffer. Reserpine and Ro 4-1284 counteract the 5HT outflux from platelets, whereas DL-amphetamine, Ro 4-6861, tyramine, and also guanethidine have no effect (Tables 2, 3). Reserpine and Ro 4-1284 seem therefore to influence the 5HT content of platelets under normal condition (e.g. in plasma) by two separate mechanisms, i.e. by releasing the amine from the storage sites and by interfering with its outflux through membranes.[1] DL-Amphetamine, Ro 4-6861, and tyramine, however, probably exert only one effect, i.e. displacement of the 5HT from the storage sites without changing the membranes. This might explain why the release of 5HT from platelets in physiological media induced by reserpine and Ro 4-1284 is slower than that caused by sympathicomimetic amines.[9]

The slight but significant attentuation of the liberation of platelet 5HT induced by the monoamine oxidase (MAO) inhibitors iproniazid and pargyline cannot be explained by interference with MAO. Thus, in glucose-free K-phosphate virtually no activity of MAO, as measured by the formation of 5-hydroxytryptophol and 5-hydroxyindoleacetic acid, could be detected. The MAO inhibitors might, however, also be able to interfere with the outflux of 5HT through platelet membranes. An action on biological membranes would, at least partly, explain the observation that some MAO inhibitors diminish the spontaneous as well as the reserpine-induced liberation of monoamines from various tissues.[10–14]

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