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BRIEF COMMUNICATION

Activation of Membrane Skeleton-Bound Phosphofructokinase in Erythrocytes Induced by Serotonin

We show here that serotonin, both *in vivo* and *in vitro,* induced a marked activation of phosphofructokinase, the rate-limiting enzyme in glycolysis, in the membrane-skeleton fraction from erythrocytes. Concomitantly, the hormone induced a striking increase in lactate content, reflecting stimulation of glycolysis. The enzyme's activity in the cytosolic (soluble) fraction remained unchanged. These results suggest a defense mechanism in the erythrocytes against the damaging effects of serotonin, whose concentration in plasma increases in many diseases and is implicated as playing an important role in circulation disturbances.

Key Words: **serotonin (5-hydroxytryptamine); phosphofructokinase; erythrocytes (red blood cells); glycolysis; cytoskeleton.**

INTRODUCTION

Plasma serotonin (5-hydroxytryptamine, 5-HT) concentration increases under many pathological conditions and diseases such as thrombosis, diabetes, arteriosclerosis, and hypertension and was recently implicated as playing an important role in circulation disturbances (1–4). 5-HT *in vitro* was reported to decrease red blood cell deformability (5).

Previous experiments in our laboratory have revealed that 5-HT exerts a deleterious effect on glycolysis in muscle and skin (6–8). We have also observed that injection of 5-HT to rats induced a marked elevation of plasma hemoglobin, reflecting lysed erythrocytes (9). These results prompted us to investigate the effect of 5-HT on glycolysis in erythrocytes. Glycolytic enzymes are known to be present in both the soluble fraction of the cell as well as bound to the cytoskeleton (10; for reviews see 11,12). In erythrocytes, glycolytic enzymes bind to band 3 (13,14), which is linked to the cytoskeleton (15). Unlike other cells where the glycolytic enzymes were shown to be activated by binding to the cytoskeleton (11,12), in erythrocytes, it has been suggested that the enzymes are inhibited upon binding to band 3 (14).

In the present study we investigated the effect of 5-HT, both *in vivo* and *in vitro,* on the activity of phosphofructokinase (PFK) (EC 2.7.1.11), the rate-limiting enzyme in glycolysis, in both the membrane-skeleton and cytosolic fractions from erythrocytes.

We report here that 5-HT induces a marked activation of PFK in the membrane-skeleton fraction which correlates with lactate production.

MATERIALS AND METHODS

Materials. 5-Hydroxytryptamine hydrochloride was purchased from Sigma Chemical Co. Other Chemicals and enzymes were either from Sigma Chemical and Co. or from Boehringer-Mannheim (GmbH).

In vivo experiments. Charles River albino rats (90–120 g body wt), fed ad libitum, were injected ip with serotonin (40 mg/kg) in 0.1 ml saline. Identical controls received 0.1 ml saline. Ten minutes later the rats were anesthetized with sodium nembutal (60 mg/kg) and blood was collected.

Preparation of erythrocytes. The blood was collected into tubes containing heparin (25 units/ml) and was held on ice for no longer than 1 h. The blood samples were centrifuged (1000*g* for 5 min)

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and the plasma and white blood cells were removed with a water jet pump. The erythrocytes were washed with 10 ml ice-cold phosphate buffer saline containing 5 mM glucose ("PBS buffer"), centrifuged as before and the supernatant was removed again. The cells were washed twice more. All the procedure was conducted at 0–4°C. The packed erythrocytes were held on ice for no longer than 1 h. Prior to the extraction of PFK or lactate, one volume of PBS buffer was added to the packed erythrocytes.

In vitro experiments. Blood from Charles River albino rats (90–120 g body wt), fed ad libitum, was used. The blood from several animals was pooled. In the experiments done with whole blood, samples (500 μl heparinized blood and 500 μl autologous plasma containing 30 μM ascorbic acid) were incubated at 37°C, in the presence or absence of 5-HT. The samples were put on ice to stop the incubation and erythrocytes were prepared as described above. In the experiments done with washed erythrocytes, the erythrocytes were prepared as described above and the suspension of erythrocytes (10% hematocrit in PBS buffer containing 30 μM ascorbic acid) was incubated at 37°C, in the presence or absence of 5-HT. After the incubation the cells were washed once more.

Separation and assay of bound and soluble PFK. Membrane-bound and soluble PFK were separated as described previously (7) with the following modification: 3 vol of extraction buffer were added to the erythrocyte solution (200 μl). The cells were homogenized for 90 s and samples (15 μl) were removed for protein determination. Extraction buffer (0.8 ml) was added to dissolve the membrane-bound fraction. PFK was assayed under maximal (optimal) conditions (pH 8.2).

Extraction and determination of lactate. Samples (5 μl) were removed for protein determination before the addition of 2.25 vol of ice-cold solution of 5% perchloric acid containing 1 mM EDTA to the erythrocyte solution (200 μl) (16). The samples were agitated vigorously and centrifuged (5000*g*). After centrifugation, the extract was neutralized with KOH and centrifuged again. The supernatant was collected for the determination of lactate with the kit from Sigma Chemical and Co.

Protein determination. Protein was measured by the method of Bradford (17) with crystalline bovine serum albumin as a standard.

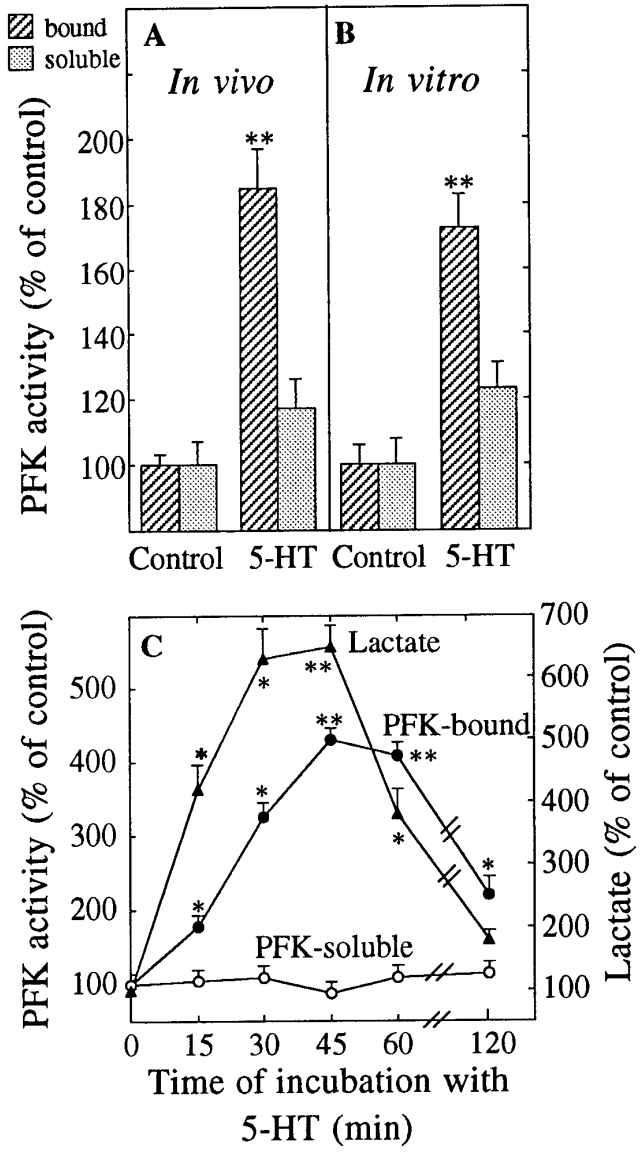

FIG. 1. Effect of 5-HT on bound and soluble PFK activity in erythrocytes under different conditions and its correlation to lactate levels. (A) *In vivo* effect of injected 5-HT. 100% refers to 1.80 ± 0.11 and 1.90 ± 0.11 munits/mg protein, for the bound and soluble PFK, respectively. (B) *In vitro* effect of 22 h incubation of whole blood with 5-HT (10^{-5} M). (C) Time–response curve of incubation of washed erythrocytes with 5-HT (10^{-2} M). 100% refers to 1.70 ± 0.21 and 1.80 ± 0.20 munits/mg protein, for the bound (●) and soluble (○) PFK, respectively, and 1.10 ± 0.12 mg lactate/mg protein (▲). Each point is the mean ± SEM for 6–12 experiments. $*P < 0.02$, $**P < 0.005$.

RESULTS

The results presented in Fig. 1A show the *in vivo* effect of 5-HT injected to rats on the membrane skel-

eton-bound and soluble PFK activity in erythrocytes. It can be seen that 5-HT induced a marked increase in the activity of the bound PFK with no effect on the soluble enzyme. Similar effect was obtained *in vitro* when 5-HT (10^{-5} M) was incubated with whole blood (Fig. 1B), or washed erythrocytes (not shown) for 22 h. With higher concentrations of 5-HT, the increase in the activity of membrane skeleton-bound PFK could also be demonstrated in short-time incubations with washed erythrocytes (Fig. 1C). The effect of 5-HT was time- and concentration-dependent. Concomitant to the increase in the activity of membrane-skeleton bound PFK induced by 5-HT, there was a marked (up to sixfold) increase in lactate production in the erythrocytes (Fig. 1C), reflecting stimulation of glycolysis.

DISCUSSION

The results reported here reveal that 5-HT induced, both *in vivo* and *in vitro*, a marked increase in the activity of PFK in the membrane-skeleton fraction from erythrocytes. Since this effect was not accompanied by a decrease in PFK activity in the cytosolic (soluble) fraction, it is unlikely that the hormone acted by translocating the enzyme from soluble to bound fraction. The results suggest that 5-HT induced an activation of preexisting membrane-skeleton bound PFK, which was reported to be inhibited upon binding to band 3 (14). It is possible that the enzyme is released from its original site of binding and thereby activated. However, since PFK activity in the soluble fraction did not rise, it seems that the enzyme remains in the particulate fraction, most probably in a different binding state and/or configuration in which it is more active. The activation of PFK cannot be attributed to allosteric effectors since the enzyme was assayed under maximal conditions in which it is not subject to allosteric regulators (7).

Concomitant to the marked activation of membrane skeleton-bound PFK, the rate-limiting enzyme of glycolysis, there was a striking increase in lactate content (Fig. 1C), reflecting stimulation of glycolysis. Previous experiments from our laboratory (9) have revealed that injection of 5-HT induces an elevation of plasma hemoglobin. Our FACS analysis of erythrocytes following incubation with 5-HT revealed marked changes in cell shape (results not shown). The present findings showing the 5-HT-induced stimulation of erythrocyte PFK and thereby glycolysis, the sole source of energy in these cells, suggest that this may be a defense mechanism against the damaging effect of this hormone.

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