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Serotonin-induced Decrease in Brain ATP, Stimulation of Brain Anaerobic Glycolysis and Elevation of Plasma Hemoglobin; the Protective Action of Calmodulin Antagonists

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Abstract—1. Injection of serotonin (5-hydroxytryptamine) to rats, induced a dramatic fall in brain ATP level, accompanied by an increase in P_i. Concomitant to these changes, the activity of cytosolic phosphofructokinase, the rate-limiting enzyme of glycolysis, was significantly enhanced. Stimulation of anaerobic glycolysis was also reflected by a marked increase in lactate content in brain.

2. Brain glucose 1,6-bisphosphate level was decreased, whereas fructose 2,6-bisphosphate was unaffected by serotonin.

3. All these serotonin-induced changes in brain, which are characteristic for cerebral ischemia, were prevented by treatment with the calmodulin (CaM) antagonists, trifluoperazine or thioridazine.

4. Injection of serotonin also induced a marked elevation of plasma hemoglobin, reflecting lysed erythrocytes, which was also prevented by treatment with the CaM antagonists.

5. The present results suggest that CaM antagonists may be effective drugs in treatment of many pathological conditions and diseases in which plasma serotonin levels are known to increase.

Key Words: Calmodulin antagonists, glycolysis, serotonin (5-hydroxytryptamine), phosphofructokinase, glucose 1,6-bisphosphate, fructose 2,6-bisphosphate, brain (cerebral) ischemia, plasma hemoglobin

INTRODUCTION

Plasma serotonin (5-hydroxytryptamine, 5-HT) levels are known to increase in many pathological conditions and diseases. For example, a 15-fold increase in plasma serotonin levels have been demonstrated during cerebrovascular thrombosis (Wester *et al.*, 1992). Elevation of plasma serotonin level by intravenous infusion of this amine into rats, significantly increased blood–brain barrier permeability and reduced cerebral blood flow (Sharma *et al.*, 1990). These findings prompted us to investigate the effects of serotonin on ATP levels and glycolysis in rat brain. Previous experiments in our laboratory have revealed that serotonin exerts a deleterious effect on muscle and skin glucose metabolism, which could be prevented by treatment with calmodulin (CaM) antagonists (Beitner *et al.*, 1982, 1983; Kaplansky and Beitner, 1984). We also found that CaM antagonists are most effective drugs for treatment of skin trauma (Beitner, 1987; Beitner *et al.*, 1989a, b, 1991) and muscle damage (Beitner and Lilling, 1993), where serotonin is known to play a pathogenic role.

We show here that injection of serotonin to rats induces in brain a dramatic decrease in ATP level, accompanied by stimulation of the activity of cytosolic phosphofructokinase (PFK), the rate-limiting enzyme of glycolysis, and accumulation of lactate. We also show that injection of serotonin causes a marked increase in plasma hemoglobin. All these pathological changes induced by serotonin were prevented by

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treatment with the CaM antagonists, trifluoperazine (TFP) or thioridazine (TRZ).

MATERIALS AND METHODS

Materials

5-hydroxytryptamine hydrochloride was obtained from Sigma Chemical Co. Trifluoperazine dihydrochloride was purchased from ICN Flow Co. and Thioridazine hydrochloride from Taro Pharmaceutical Co., Inc., Haifa, Israel. Other chemicals and enzymes were either from Sigma Chemical Co. or from Boehringer Mannheim GmbH.

Injection of serotonin and CaM antagonists

Charles River albino rats (90–120 g) fed *ad libitum* were injected i.p. with serotonin (40 mg/kg) in 0.1 ml saline. Identical controls received 0.1 ml saline. 10 min later the rats were anesthetized with sodium nembutal (60 mg/kg) and the brains were removed.

In the experiments in which effects of CaM antagonists were studied, trifluoperazine (TFP) or thioridazine (TRZ) at a concentration of 80 mg/kg body wt in 0.1 ml water were injected i.p. 100 min prior to injection of serotonin.

Brains were removed and rapidly frozen between a pair of aluminum tongs precooled in liquid N_2 and stored in liquid N_2 until used (24 hr). Frozen brains were powdered in a mortar cooled in liquid N_2. The powder was used for extraction of metabolites and enzymes.

Preparation and assay of bound and soluble PFK

Cytoskeleton-bound and soluble PFK were separated as described previously (Lilling and Beitner, 1990; Lilling *et al.*, 1991). PFK was assayed under maximal (optimal) conditions (pH 8.2), as described by Mansour (1972). PFK was also assayed under allosteric (suboptimal) conditions (pH 7.35), in which it is subject to allosteric regulation, in an assay mixture similar to that described by Mansour (1972), except that ATP concentration was 0.5–1 mM. One unit of PFK activity catalyzed the formation of 1 μmol of fructose 1,6-bisphosphate per min at 25°C.

Extraction and determination of metabolites

All metabolites except fructose 2,6-bisphosphate (Fru-2,6-P_2) were extracted in 2 ml perchloric acid as described previously (Beitner *et al.*, 1978). Glucose 1,6-bisphosphate (Glc-1,6-P_2) was measured by the fluorometric method of Passonneau *et al.* (1969). ATP and inorganic phosphate were measured by the methods of Lowry *et al.* (1964). Lactate was measured with the kits from Sigma Chemical Co. Fru-2,6-P_2 was extracted and assayed by the method described by Van Schaftingen *et al.* (1982) with the modification introduced by Van Schaftingen and Hers (1983).

Hemoglobin determination

Hemoglobin in plasma was measured with the kit no. 527 from Sigma Chemical Co.

Protein determination

Protein was measured by the method of Lowry *et al.* (1951) with crystalline bovine serum albumin as a standard.

Statistical analysis

Experimental results are expressed as mean ± SE. Student's t-test was used to determine statistical significance of differences between groups; $P < 0.05$ was considered significant.

RESULTS

The experiments presented in Table 1 reveal that injection of serotonin to rats induced a drastic fall in the level of brain ATP (to 24% of normal level), accompanied by an increase in P_i. The levels of Glc-1,6-P_2, the potent regulator of carbohydrate metabolism (for reviews, see Beitner, 1979, 1984, 1985, 1990, 1993), were significantly decreased, whereas Fru-2,6-P_2 remained unchanged. Lactate content was markedly (more than 3-fold) elevated, suggesting stimulation of glycolysis.

Table 2 shows the effect of serotonin on brain cytoskeleton-bound and soluble (cytosolic) PFK, the rate-limiting enzyme of glycolysis. The allosteric activity was assayed under regulatory (suboptimal) conditions (pH 7.35), in which the enzyme is subject

Table 1. Effects of serotonin on the levels of ATP, Pi, Glc-1,6-P_2, Fru-2,6-P_2 and lactate in brain

Conditions	ATP (mmol/kg)	Pi	Glc-1,6-P_2 (μmol/kg)	Fru-2,6-P_2 (μmol/kg)	Lactate (mg/g)
Control	1.24 ± 0.24	5.89 ± 0.81	84.0 ± 6.1	7.34 ± 1.05	0.39 ± 0.16
Serotonin	0.30 ± 0.11	9.54 ± 1.00	46.7 ± 12.7	6.74 ± 0.89	1.22 ± 0.07
% change	−76	+62	−44	—	+213
P value	$P < 0.005$	$P < 0.005$	$P < 0.005$	N.S.	$P < 0.005$

Values are means ± SE for 6–12 experiments.
N.S., not significant.

Table 2. Effects of serotonin on cytoskeleton-bound and soluble PFK in brain

	PFK allosteric activity (units/g)		PFK maximal activity (units/g)	
Conditions	Cytoskeleton-bound	Soluble	Cytoskeleton-bound	Soluble
Control	0.27 ± 0.13	1.65 ± 0.21	2.18 ± 0.25	13.33 ± 0.72
Serotonin	0.29 ± 0.08	3.60 ± 0.60	2.32 ± 0.27	12.33 ± 1.36
% change	—	+118%	—	—
P value	N.S.	$P < 0.005$	N.S.	N.S.

Values are means ± SE for 6–12 experiments.
N.S., not significant.

to regulation by its allosteric effectors, and the maximal activity was assayed under maximal (optimal) conditions (pH 8.2), in which the enzyme is not sensitive to its allosteric effectors. It can be seen that injection of serotonin induced a significant enhancement of the allosteric activity of soluble but not cytoskeleton-bound PFK. The maximal activity of the enzyme remained unchanged in both cell fractions. These results reveal that serotonin did not affect the intracellular distribution of PFK, and the marked stimulation of cytosolic PFK resulted most probably from changes in the concentration of its allosteric effectors.

In the experiments presented in Figs 1–3 we studied whether treatment with the CaM antagonists, trifluoperazine (TFP) or thioridazine (TRZ), would prevent the pathological changes induced by serotonin. It can be seen that both CaM antagonists reversed the pathological decrease in ATP [Fig. 1(a)] as well as the increase in Pi [Fig. 1(b)], to normal control levels. These compounds also prevented the increase in soluble PFK activity [Fig. 2(a)] and lactate

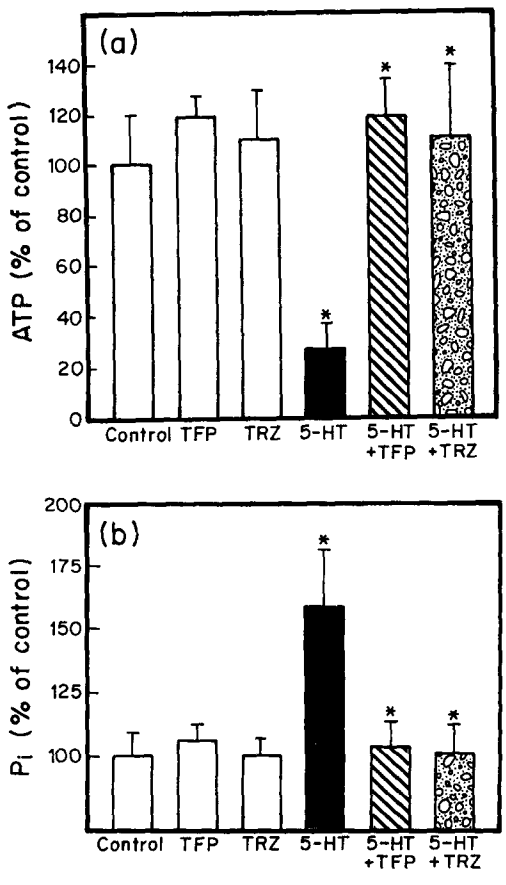

Fig. 1. The antagonistic effect of CaM inhibitors to the action of serotonin on ATP (a) and P_i (b) levels in brain. Values are mean ± SE for 6–12 experiments. $*P < 0.005$ (5-HT vs control, or 5-HT + CaM inhibitor vs 5-HT).

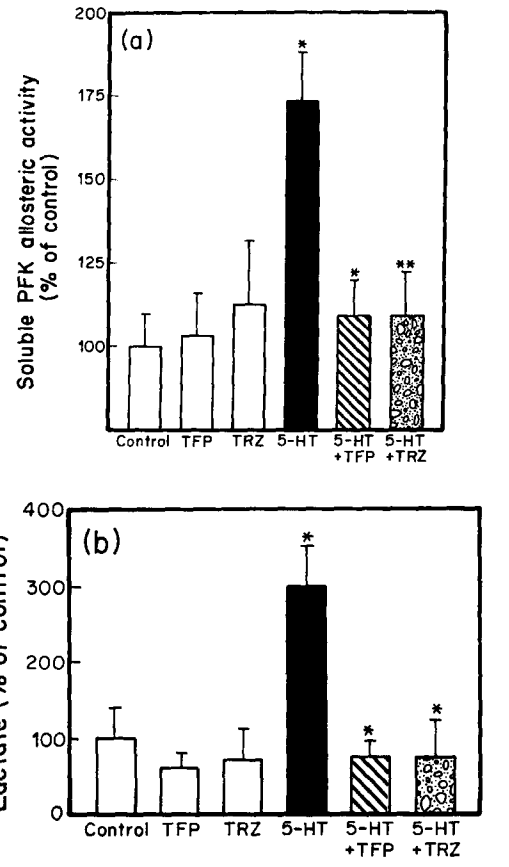

Fig. 2. The antagonistic effect of CaM inhibitors to the action of serotonin on soluble PFK allosteric activity (a) and lactate levels (b) in brain. Values are mean ± SE for 6–12 experiments. $*P < 0.005$; $**P < 0.05$ (5-HT vs control, or 5-HT + CaM inhibitor vs 5-HT).

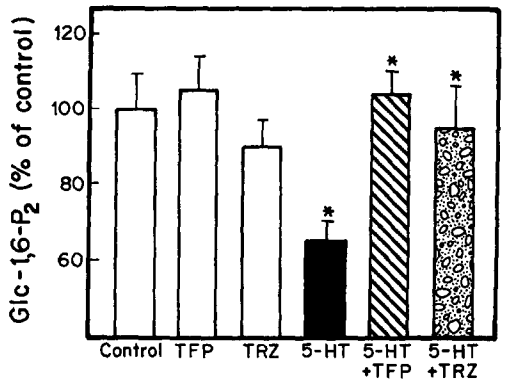

Fig. 3. The antagonistic effect of CaM inhibitors to the action of serotonin on Glc-1,6-P_2 levels in brain. Values are mean ± SE for 6–12 experiments. *$P < 0.005$ (5-HT vs control, or 5-HT + CaM inhibitor vs 5-HT).

content [Fig. 2(b)]. The CaM antagonists also reversed the reduction in Glc-1,6-P_2 level induced by serotonin (Fig. 3). The CaM antagonists alone had no effect on any of the parameters studied.

During these studies, we observed that injection of serotonin causes blood hemolysis. We therefore measured plasma hemoglobin obtained from rats following the injection of serotonin. As shown in Fig. 4, injection of serotonin induced a marked (about 5-fold) increase in plasma hemoglobin. This increase was prevented by treatment with TFP or TRZ. The CaM antagonists alone had no effect on plasma hemoglobin.

DISCUSSION

The present results reveal that injection of serotonin to rats induces a dramatic fall in ATP level in brain, accompanied by an increase in P_i (Table 1).

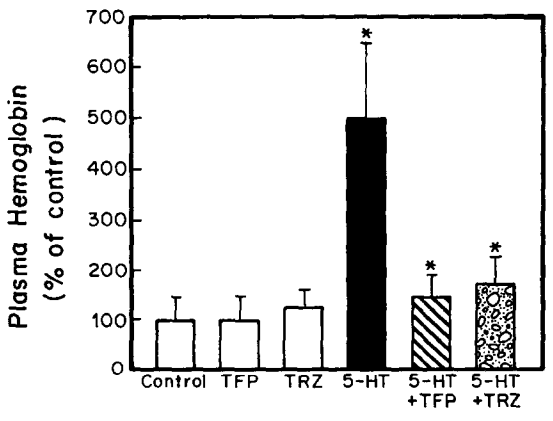

Fig. 4. The antagonistic effect of CaM inhibitors to the action of serotonin on plasma hemoglobin levels. Values are mean ± SE for 6–12 experiments. *$P < 0.005$ (5-HT vs control, or 5-HT + CaM inhibitor vs 5-HT).

Concomitant to the decrease in ATP, which is an allosteric inhibitor of PFK, and the increase in P_i an allosteric activator of this enzyme, the allosteric activity of PFK in cytosol was markedly enhanced (Table 2). In contrast to cytosolic (soluble) PFK, the allosteric activity of cytoskeleton-bound enzyme was not affected by serotonin, since it is less sensitive to allosteric inhibition by ATP and activation by P_i (results not shown). The enhancement of cytosolic PFK, the rate-limiting enzyme of glycolysis, leads to stimulation of glycolysis, which is reflected by the marked increase in lactate (Table 1).

The serotonin-induced changes in ATP, P_i, PFK and glycolysis, as well as the reduction in Glc-1,6-P_2, are typical changes which we found to occur in tissue during anoxia (Beitner *et al.*, 1979). Serotonin was reported to induce cerebral arteriolar constriction (Thompson *et al.*, 1984). It also increases blood–brain barrier permeability and reduces regional cerebral blood flow (Olesen, 1985; Sharma *et al.*, 1990). Through these actions, serotonin may induce brain ischemia, which is reflected here by the fall in ATP and the stimulation of anaerobic glycolysis, changes that are characteristic for cerebral ischemia (Sims, 1992). Serotonin has been implicated in the pathogenesis of neuronal damage during ischemia (Globus *et al.*, 1992). Serotonin or ischemia is known to induce accumulations of intracellular calcium which causes cell damage. It was recently found that the calmodulin antagonists, phenothiazines, reduce brain damage after ischemia (Zivin *et al.*, 1989; Yu *et al.*, 1992). Calmodulin antagonists are also cardioprotective in ischemic heart, preserving myocardial ATP (Sargent *et al.*, 1992). We show here that the changes induced by serotonin in brain ATP, P_i, PFK, lactate and Glc-1,6-P_2, were prevented by treatment with the calmodulin antagonists, TFP or TRZ (Figs 1–3).

An interesting observation is the increase in plasma hemoglobin induced by serotonin (Fig. 4). This increase, which reflects lysed erythrocytes, is compatible with the *in vitro* experiments, which have shown that exposure of erythrocytes to serotonin increases their osmotic fragility (Kirshtein and Gilboa-Garber, 1975). The increase in plasma hemoglobin may be a useful parameter to measure, in various pathological conditions in which plasma serotonin concentration is increased. The calmodulin antagonists, TFP or TRZ, prevented the serotonin-induced elevation in plasma hemoglobin (Fig. 4), suggesting a protective action on erythrocyte membrane against the damaging effect of serotonin. We have also previously found that serotonin induces pathological changes in glucose metabolism in muscle and skin, which were prevented by treatment with calmodulin antagonists (Beitner *et al.*, 1982, 1983;

Kaplansky and Beitner, 1984). Calmodulin antagonists were also found in our laboratory to be effective drugs in treatment of skin injuries, e.g. burns or frostbite (Beitner, 1987; Beitner *et al.*, 1989a, b, 1991), as well as in treatment of muscle damage (Beitner and Lilling, 1993). It has recently become evident that calmodulin antagonists are a new generation of drugs with broad therapeutic applications (for review, see Mannhold and Timmerman, 1992).

The present findings which have revealed that calmodulin inhibitors antagonize the pathological effects of serotonin in brain, as well as the serotonin-induced raise in plasma hemoglobin, suggest that these compounds may be effective drugs in various pathological conditions and diseases in which plasma serotonin levels are increased.

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