BBA 63333

The effect of 5-hydroxytryptamine on glycogen phosphorylase

The stimulation exerted by 5-hydroxytryptamine on carbohydrate metabolism has been studied by several authors. The data found in the literature show that there are some variations depending on the animal species examined, but in most cases the effects resulted in hyperglycaemia or in an increase of glycogenolysis. 5-Hydroxytryptamine induced hyperglycaemia in mice and rabbits, while hypoglycaemia and an increase of the blood lactic and pyruvic acid contents were observed in rats after administration of 5-hydroxytryptamine[1]. In the liver fluke, *Fasciola hepatica*, 5-hydroxytryptamine was able to stimulate synthesis of cyclic 3',5'-AMP. Consequent activation of phosphofructokinase was also observed[2]. The hyperglycaemia induced by 5-hydroxytryptamine in dogs was accompanied by a decrease of the liver glycogen content and an increase of the phosphorylase activity. Since these effects were abolished by adrenalectomy, it was supposed that the catecholamines were responsible for the glucose metabolism[3]. 5-Hydroxytryptamine added to rat adipose tissue increased the phosphorylase activity *in vitro*, suggesting that 5-hydroxytryptamine may also have a direct effect on the enzyme[4].

The present paper deals with the direct interaction *in vitro* between the glycogen phosphorylase (α-1,4-glucan:orthophosphate glycosyltransferase, EC 2.4.1.1) and 5-hydroxytryptamine–creatinine sulphate. The effect of 5-hydroxytryptamine has been

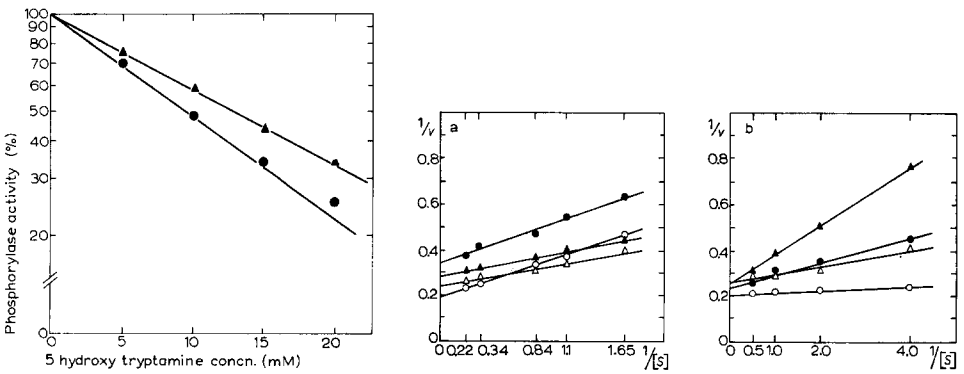

Fig. 1. Phosphorylase activity as a function of 5-hydroxytryptamine concentration. ●—●, $4 \cdot 10^{-8}$ M phosphorylase *a* in 0.05 M Tris–HCl buffer at pH 6.8; ▲—▲, $8 \cdot 10^{-8}$ M phosphorylase *b* in 0.05 M Tris–HCl buffer at pH 6.8. The reaction mixture contained 0.2 ml enzyme, 0.1 ml 5-hydroxytryptamine–creatinine sulphate (25, 50, 75, 100 mM) and 0.2 ml substrate (32 mM Glc-1-*P*, 2 mM AMP, 2% glycogen).

Fig. 2.a. Effect of 5-hydroxytryptamine on the phosphorylase activity as a function of the Glc-1-*P* concentration. The kinetic parameters were obtained from $1/v$ plots according to Lineweaver and Burk. Five concentrations of Glc-1-*P* were used in the range 6–48 mM. The other substrate constituents were unchanged. The reaction mixture contained 0.2 ml enzyme, 0.1 ml 5-hydroxytryptamine–creatinine sulphate and 0.2 ml substrate. The solid symbols indicate the presence of 5-hydroxytryptamine. b. Effect of 5-hydroxytryptamine on the phosphorylase activity as a function of the AMP concentration. Four concentrations of AMP were used in the range 0.25–2.0 mM. The other substrate constituents were unchanged. The solid symbols indicate the presence of 5-hydroxytryptamine. 0.1 ml of 25 mM 5-hydroxytryptamine present in both a and b. Enzyme concns. as in Fig. 1.

investigated on phosphorylase *b* as well as on phosphorylase *a*. It is well known that the former consists of two subunits and requires the presence of AMP for enzyme activity, while the *a* form is composed of four subunits and contains four phosphoseryl residues. Phosphorylase *a* demonstrates 60–70% of its full activity without AMP, very likely due to the phosphoseryl residues.

Phosphorylase *b* was prepared from rabbit muscle according to the procedure of FISCHER AND KREBS[5,6], while phosphorylase *a* was produced from 4 × recrystallized phosphorylase *b* by using phosphorylase kinase and ATP[5,6]. The crystalline enzymes were further purified by passing them through a column of Sephadex G-100 immediately before use. The gel column was equilibrated with 0.05 M Tris–HCl buffer at pH 6.8. The activity assay as well as the specific activity determinations were carried out in the direction of glycogen synthesis as described earlier[7]. 5-Hydroxytryptamine–creatinine sulphate, DL-5-hydroxytryptophan, tryptamine and 5-hydroxyindole-acetic acid (5–20 mM end concn.) were added to the reaction mixture of the enzyme and substrate.

The loss in activity of phosphorylase *a* and *b* is shown in Fig. 1 plotted against the concentration of 5-hydroxytryptamine. An exponential relationship was found between the loss of activity and 5-hydroxytryptamine concentration, which also indicated the binding ability of 5-hydroxytryptamine to the inactivated molecules. The activity of phosphorylase *a* was affected to a somewhat greater extent than that of phosphorylase *b*.

Table I demonstrates the effects of 5-hydroxytryptamine and some indole derivates in order to eliminate the possibility of the unspecific effect of creatinine sulphate, which is present in the 5-hydroxytryptamine preparation. As may be seen from the data, inhibition took place only in the presence of an indole ring.

Further experiments were carried out in order to study the mechanism of the effects of 5-hydroxytryptamine described above. Fig. 2 shows the interaction of different concentrations of substrate (Glc-1-*P*) and 5-hydroxytryptamine on the phosphorylase *a* and *b*. The Lineweaver–Burk plot clearly demonstrates the non-competitive effect, *i.e.* the binding of substrate and 5-hydroxytryptamine were independent. Fig. 2b shows the combined effect of 5-hydroxytryptamine and of allo-

TABLE I

EFFECTS OF 5-HYDROXYTRYPTAMINE AND SOME INDOLE DERIVATES ON THE PHOSPHORYLASE ACTIVITY

The enzyme activity is expressed in μg P_i liberated per min.

Indole derivatives	*Control*		*Inhibitor*							
			5 mM		*10 mM*		*15 mM*		*20 mM*	
Phosphorylase:	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
5-Hydroxytryptamine–creatinine sulphate	36.5	40.0	25.5	30.0	17.2	23.5	12.5	17.5	9.8	13.6
DL-5-Hydroxytryptophan	37.0	39.5	23.0	28.0	15.0	21.5	11.0	15.0	7.0	10.0
Tryptamine	36.5	41.0	20.0	34.0	16.0	23.0	11.0	13.0	9.0	9.0
5-Hydroxyindole-3-acetic acid	36.5	41.0	22.5	30.5	16.0	20.0	10.0	12.5	7.0	9.5
Creatinine	37.0	40.0	36.5	40.0	36.5	39.0	36.5	39.0	36.5	39.5

steric activator AMP on the activity of glycogen phosphorylase. The intersection of the curves on the $1/v$ axis demonstrates the competitive inhibition between AMP and 5-hydroxytryptamine in connection with the phosphorylase *b*. The inhibition of phosphorylase *a* proved to be of a mixed type.

The results suggest that phosphorylase *b* is inhibited by the stabilization of an inactive conformation, according to allosteric theory. The effect of 5-hydroxytryptamine on phosphorylase *a* seems to be a little more complicated. This form of the glycogen phosphorylase has no absolute requirement for AMP and inhibition was not competitive with AMP. We suggest an indirect interaction, very likely due to a conformational change in the enzyme structure. The strong electronic field of the 5-hydroxytryptamine molecule makes this suggestion plausible.

Comparing the above findings to the data of other authors, we concluded that it is impossible to increase the glycogen mobilisation by 5-hydroxytryptamine directly, because of the inhibiting effect of 5-hydroxytryptamine on the key enzyme of glycogenolysis. The following scheme is proposed for the indirect effect of 5-hydroxytryptamine:

5-Hydroxytryptamine → adrenal gland → adenyl cyclase → 3′,5′-AMP → active kinase →
→ active phosphorylase → increased glycogenolysis

The experimental data which support this scheme will be presented elsewhere.

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Enzymic basis for a genetic suppression: Accumulation and deacylation of *N*-acetylglutamic γ-semialdehyde in enterobacterial mutants

Double mutants[1] of *Escherichia coli* W, blocked in the path of proline synthesis (before glutamic γ-semialdehyde) and in the path of arginine synthesis at acetylornithine δ-transaminase[2] (α-*N*-acetyl-L-ornithine:2-oxoglutarate aminotransferase, EC 2.6.1.11) grow slowly on minimal medium. It was suggested[1] that growth without