ACTIVATION OF ADENYL CYCLASE WITH HEXOSE MONOPHOSPHATES

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SUMMARY: Adenyl cyclase activity from rat kidney was stimulated with glucose 1-phosphate, glucose 6-phosphate, fructose 1-phosphate and fructose 6-phosphate, but fructose 1,6-diphosphate and glucose 1,6-diphosphate did not exerted any activation of the adenyl cyclase activity.
These facts may suggest possible "feed back" relation between a rate limiting enzyme in glycolytic system.

INTRODUCTION: It has been already established well that adenosine 3'5'-cyclic monophosphate (cAMP) stimulates glycogenolysis (1), as well as many array of cell functions (2). Hence, it might be interesting to investigate whether some glycolytic intermediates would stimulate adenyl cyclase activity, since activating effect of glycolytic intermediates, if it were, should suggest "feed back" regulation of adenyl cyclase with glycolytic system.

The present paper deals with effect of glucose 1-phosphate (G1P), glucose 6-phosphate (G6P), fructose 1-phosphate (F1P), fructose 6-phosphate (F6P), glucose 1,6-diphosphate (GDP) and fructose 1,6-diphosphate (FDP) on adenyl cyclase activity in the partially purified rat kidney plasma membrane.

MATERIALS and METHODS: All experiments were carried out on the

partially purified plasma membrane fraction of rat kidney prepared according to the procedure of Marx et al '72 (3), with a slight modification.

Adenyl cyclase activity was estimated with the determination of cAMP produced after an incubation of 0.6 ml mixture containing 25 mM tris-HCl at pH 7.6, 40 mM phosphoenolpyruvate (PEP), 5 mM caffeine, 5 mM $MgCl_2$, 2.5 mM ATP, 3 µg pyruvatekinase (PK) and the plasma membrane suspension (about 1 mg per each of the mixture). The mixture also contained, if necessary, G1P, G6P, F1P, F6P, GDP and FDP at a concentration running from 10^{-7} M to 10^{-4} M respectively.

The enzyme reaction was started by pouring of the plasma membrane suspension and stopped with addition of 1 ml of ice cold 0.5 M perchloric acid into the mixture after 5 minutes' incubation at 37°C. After a centrifugation at 15,000 xg for 10 minutes in the cold, the aliquot obtained was neutralized with saturated K_2CO_3 in an ice bath, and the heaby precipitate thus formed was eliminated with the centrifugation. The aliquot finally obtained was analyzed for cAMP with the method of Gilman (4). Adenyl cyclase activity was expressed as p moles cAMP produced/minute/mg protein. Estimation of protein were performed according to the method of Lowly et al (5).

G1P, G6P, F1P, F6P, GDP, FDP, ATP, cAMP, PEP and PK were the products of Boelinger Manheim Co. West Germany, and ^{3}H-cAMP (25 Ci/m mole) was obtained from New England Nuclear Corp. All other chemicals were the reagent grade.

RESULTS: As shown in Table 1, adenyl cyclase from rat kidney was enhanced significantly with G1P, G6P, F1P and F6P respectively. F6P enhanced adenyl cyclase in the highest degree among

	% activation of adenyl cyclase	
	10^{-6}M	10^{-5}M
G1P	59.5 ± 32.4	117.0 ± 18.9
G6P	61.9 ± 9.0	68.9 ± 7.5
F1P		109.5 ± 20.5
F6P	107.9 ± 11.1	132.7 ± 11.1
GDP	1.2 ± 11.4	-11.9 ± 18.4
FDP	8.6 ± 3.2	-1.4 ± 9.2

Table 1. Percent activation of adenyl cyclase with hexose phosphates. Each value represents average of 4 experiments ± S.E.

hexose phosphates examined. FDP and GDP failed to stimulate the enzyme activity. It is assumed that fructose and glucose monophosphate esters stimulate adenyl cyclase activity, but diphosphate esters can not enhance its activity.

Fig 1 also shows stimulating effect of hexose phosphates on adenyl cyclase activity. Maximum activity of adenyl cyclase was observed in the presence of F6P at 10^{-5} M, and the activity decreased at a concentration above 10^{-4} M. Adequate concentrations of hexose monophosphates for activation of adenyl cyclase were 10^{-5} M for G1P and F1P, and 10^{-6} M for G6P respectively. F1P or G1P failed to stimulate further the activity of adenyl cyclase which had been activated with 10^{-6} M F6P (Data is not shown). FDP as well as GDP did not enhance the enzyme activity as far as examined, as also shown in Fig 1.

Activating effect of F6P on adenyl cyclase activity was also found even in the presence of 10 mM NaF, as shown in Fig 2. Since the additional increase in the enzyme activity with F6P in the presence of NaF was very similar in its value to that found without NaF, the mechanism for the enzyme activation with NaF may be different from that with hexose monophosphates. Hexose

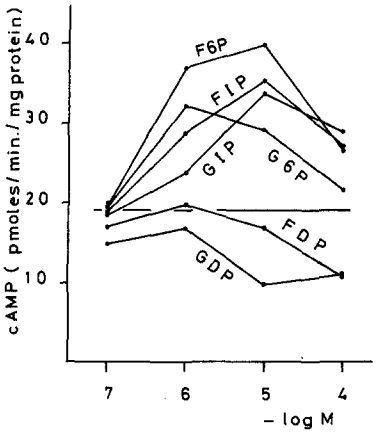

Fig. 1. Activation of adenyl cyclase with hexose phosphates.

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diphosphates did not activate the enzyme activity even in the presence of NaF.

DISCUSSION: The activation of adenyl cyclase with hexose monophosphates is not so high as found with NaF. However, the mechanism of adenyl cyclase activation with hexose monophosphates seems to be different from that with NaF, because hexose monophosphates activate the enzyme activity additionally even in the presence of NaF. Since the activating mechanism for adenyl cyclase with peptide hormones has been suggested to be the same one as NaF-activation (6), hexose monophosphate activation of the enzyme may be the other controlling mechanism for adenyl cyclase activity than that with hormones. Moreover, it can be assumed that activation of adenyl cyclase with hexose monophosphate occures even in intact cells and takes a part in the regulation of cAMP production, since the concentrations of hexose monophosphates are supposed to be in the ranges of intracellular concen-

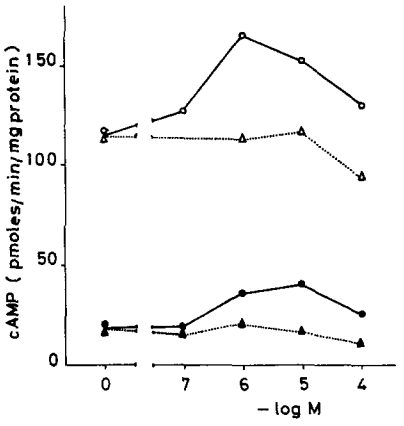

Fig. 2. Effect of 10 mM NaF on adenyl cyclase activated with F6P. —o— : 10^{-5} M F6P + 10 mM NaF, ·····Δ····· : 10^{-5} M FDP + 10 mM NaF, —●— : 10^{-5} M F6P, ·····▲····· : 10^{-5} M FDP.

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trations of hexose monophosphates in the mammalian tissues (7).

A role of hexose-monophosphate-activation of adenyl cyclase in the regulation of cell function may be that concerned with glycolysis. In the present study, F6P was found to be the most effective for adenyl cyclase activation among these hexose monophosphates examined. If phosphofructokinase (PFK) which is one of the rate-limiting enzymes for glycolysis, is very low in its activity, intracellular concentrations of hexose phosphates will increase probably to a level at which adenyl cyclase is stimulated. Thus, level of cAMP will increase and it stimulates PFK activity, since it has been well known that cAMP enhances PFK activity (8). Therefore, it is probable that PFK is also enhanced, although indirectory, with F6P level in the cells. If PFK will be activated thus, G1P, G6P and F6P in the cell may be easily converted to FDP, since there is no limiting step in glycolytic pathway from G1P to F6P. Then, adenyl cyclase activity

will decrease to an inactive state, if there is no other activator in the cell.

Therefore, it is probable that "feed back" mechanism also operates between adenyl cyclase and PFK, which is one of glycolytic key enzymes by the aid of cAMP, the product of adenyl cyclase, and F6P, a substrate of PFK.

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