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SHORT COMMUNICATIONS

Stimulatory effect of ATP on the activity of adenylate cyclase

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Various reports have suggested the possible stimulatory effects of ATP, through the guanine nucleotide regulatory protein [1–3]. More recent data, however, seem to indicate that the stimulatory effect of ATP could be due to contaminant GTP [4]. It is known from the literature [5] and we have confirmed (unpublished observation) that preactivation of the adenylate cyclase system by Gpp (NH)p is inhibited by GTP. It follows that if preactivation by Gpp (NH)p were stimulated by ATP, this phenomenon would represent a mechanism distinct from that of GTP. In the subsequent work we followed this idea.

ATP, creatine phosphate, creatine phosphokinase, Gpp (NH)p and sodium fluoride were purchased from Sigma (St. Louis, MO, U.S.A.). [α-^{32}P]ATP (23 Ci/mmole) was obtained from the Radiochemical Centre (Amersham U.K.). [8-^{14}C]GTP (60 mCi/mmole) was from New England Nuclear, Boston, MA, U.S.A. Neutral aluminiumoxid was bought from Merck, Darmstadt, F.R.G. 2-deoxyadenosine was a gift from Boehringer Mannheim, F.R.G.

Liver plasma membranes were prepared from female PVG/c rats (6-8 weeks old) according to the method of Neville [6]. In the experiments, the assay mixture for the measurement of adenylate cyclase activity contained 0.5 mM [α-^{32}P]ATP (25 μCi/μmole), 4 mM $MgCl_2$, 1 mM cyclic AMP, 15 mM creatine phosphate, 30 μg creatine phosphokinase, 30 mM Tris–HCl pH 7.5 and 50 μg protein of preincubated liver plasma membrane in a final volume of 70 μl. Incubations were performed at 33° for various lengths of time and were terminated according to the method of White [7]. Cyclic AMP was separated by column chromatography on neutral alumina [7] using 3 ml of 10 mM imidazole buffer pH 7.5 for elution.

Protein was determined according to the method of Lowry *et al.* [8] using bovine serum albumin as standard.

In preliminary experiments we established that 15 min preincubation of plasma membrane at 33° with 10^{-4}M Gpp(NH)p or 10 mM fluoride was required for maximum stimulation of adenylate cyclase activity by these activators alone.

The presence of Mg^{2+} significantly increased the preactivatory effects of both flouride and Gpp(NH)p (Fig. 1 A, B) which is in agreement with data in the literature [9]. In the absence of Mg^{2+}, ATP at 2 mM concentration strongly inhibited the activatory effect of fluoride. The inhibitory effect of uncomplexed ATP on fluoride activation has been previously observed on the cyclase activities of brain [10] and fat cell [11].

In the presence of Mg^{2+}, ATP further enhanced the preactivatory effects of both flouride and Gpp(NH)p (Fig.

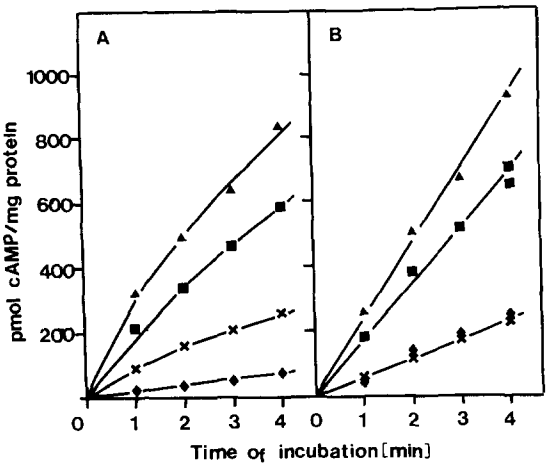

Fig. 1. Effect of preincubation of plasma membrane with 2 mM ATP on the activation of adenylate cyclase by fluoride and Gpp (NH)p. Plasma membrane proteins (1050 μg) were preincubated for 12 min at 33° in 1 ml volume containing 25 mM Tris–HCl and, if present, 10 mM sodium fluoride, 0.1 mM Gpp(NH)p, 2 mM ATP and 4 mM $MgCl_2$. After centrifugation for 3 min at 17,000 *g* and two washings with 20 mM Tris buffer, the final pellet was resuspended in Tris buffer and used immediately. Each point represents the mean value of triplicate determinations in one experiment. Similar results were obtained with two other plasma membrane preparations (A) Preincubation was performed with fluoride without further addition (x-x), with ATP (◆-◆), with $MgCl_2$ (■-■) and with ATP + $MgCl_2$ (▲-▲). (B) Preincubation was performed with Gpp (NH)p without further additions (x-x), with ATP (◆-◆), with $MgCl_2$ (■-■) and with ATP + $MgCl_2$ (▲-▲).

Table 1. Effect of preincubation of plasma membranes with 0.5 mM ATP on the activation by fluoride and Gpp(NH)p*

Additions for preincubation	pmoles Cyclic AMP/mg protein/5 min Activator			
	Gpp(NH)p	P	Fluoride	P
None	143 ± 16		221 ± 10	
ATP 0.5 mM	168 ± 12	n.s.	203 ± 19	n.s.
$MgCl_2$ 4 mM	254 ± 18	<0.01	460 ± 22	<0.01
ATP 0.5 mM + $MgCl_2$ 4 mM	325 ± 8	<0.01	556 ± 24	<0.01

* Conditions for the preincubation experiments were the same as described in the Legend to Fig. 1. except that the time of preincubation was 5 min and 0.5 mM ATP was used. Activators were added only for preincubation. Basal activity with preincubated membrane was 34 pmoles cyclic AMP/mg protein/5 min. Results are the mean ± S.E.M. of four independent experiments each performed in quadruplicate with the same plasma membrane preparation.

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1 A, B). Activatory effects of ATP of the same magnitude were obtained when plasma membranes were preincubated for 5 min in the presence of activators, Mg^{2+}, and only 0.5 mM ATP (Table 1).

Creatine phosphate, 15 mM in the absence of Mg^{2+}, inhibited the activation of adenylate cyclase by either activator. In the presence of 4 mM $MgCl_2$, we did not observe any inhibitory effect of creatine phosphate. In the next preincubation experiments we used regenerating system and established that as low as 0.1 mM concentration of ATP caused 20 per cent increase of the fluoride or Gpp(NH)p preactivation in the presence of 4 mM Mg^{2+}, (preincubation time 5 min).

The effect of ATP + Mg^{2+} was not diminished by 2-deoxyadenosine, a potent inhibitor of the phosphorylation of liver plasma membrane proteins (unpublished data). Therefore, this ATP effect is unlikely to be linked to phosphorylation.

Due to the action of nucleotide pyrophosphatase and 5′ AMP nucleotidase of plasma membrane, a significant amount of adenosine is formed from ATP during incubation [12]. Adenosine, tested up to 0.5 mM, did not mimic the stimulatory effect of ATP.

The effect of ATP, in principle, could be due to contaminant GTP [4]. GTP, (10 μM) however, reduced the Gpp(NH)p activation by 30 per cent. The possible reason for this inhibition is that GTP is hydrolysed to GDP and the latter also binds to the guanine nucleotide binding protein keeping the cyclase system in the low activity state [13–15]. GTP did not significantly modify the preactivatory effect of fluoride.

In summary, present data indicate that the Mg^{2+}-complexed ATP, besides its substrate role, has a secondary stimulatory effect on the adenylate cyclase activity. This effect is observed at concentrations which are generally used for adenylate cyclase assay. The following mechanisms and artefacts, which could explain this effect of ATP, were ruled out: (1) suspension of the inhibitory effect of creatine phosphate by ATP; (2) stimulation by adenosine or GTP instead of ATP; and (3) the role of phosphorylation. The site of the ATP effect may be the regularory protein or the complex of the catalytic unit and regulatory protein. Since guanine nucleotides and fluoride act through a common regulatory protein [16–18], it is not surprising that Mg^{2+}-complexed ATP influences their effects similarly. The regulatory protein also participates in the hormone stimulation of the cyclase system [19]. Therefore, it is likely that the observed ATP effect is a general phenomenon. Further work on the clarification of this latter point and on the exact mechanism of ATP effect is in progress in our laboratory.

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Abolition of the apparent deficiency of 2-aminophenol glucuronidation in perfused Gunn rat liver by pentan-3-one

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A wide variety of endogenous and exogeneous compounds including drugs, xenobiotics and carcinogens are conjugated with glucuronic acid by microsomal UDP-glucuronosyltransferase (EC 2.4.1.17) prior to excretion and elimination from mammals [1]. Our current knowledge of the regulation of UDP-glucuronyltransferase activity *in vivo* is limited and study of the genetic deficiency of this enzyme provides a good opportunity to examine this problem.

In 1938, Gunn described a mutant strain of Wistar rat which exhibits hereditary hyperbilirubinaemia [2]. UDP-glucuronosyltransferase activity has been shown to be defective in Gunn rat liver, and it has been shown that biochemical lesion in Gunn rat liver results in the complete inability to glucuronidate bilirubin *in vivo* or *in vitro* [3, 4]. This transferase deficiency is also recognised by a very poor ability to glucuronidate 2-aminophenol *in vitro* [5, 6]; however, addition of diethylnitrosamine [7] or alkyl ketones [8, 9] to Gunn rat liver homogenates *in vitro* surprisingly raised the deficient UDP-glucuronosyltransferase activity towards 2-aminophenol up to the activity levels in similarly-treated Wistar rat liver homogenates, such that this deficiency of UDP-glucuronosyltransferase activity was no longer apparent.

It is important to compare the regulation of UDP-glu-