P₂-purinergic control of liver glycogenolysis

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Purinergic agonists cause a dose-dependent activation of glycogen phosphorylase in isolated rat hepatocytes. Half-maximally effective concentrations are 5×10^{-7} M for ATP, 2×10^{-6} M for ADP, and about 5×10^{-5} M for AMP and adenosine. This potency series indicates the presence of P₂-purinergic receptors. The mode of action of ATP appears to be identical with that of the Ca²⁺-dependent glycogenolytic hormones angiotensin, vasopressin and α_1 -adrenergic agonists. (1) They all require Ca²⁺ for phosphorylase activation; (2) they do not increase cyclic AMP levels; (3) they are susceptible to heterologous desensitization by vasopressin and phenylephrine; (4) they lower cyclic AMP concentrations in hepatocytes stimulated by glucagon, most probably mediated by an enhanced phosphodiesterase activity.

INTRODUCTION

The cyclic AMP-independent stimulation of glycogenolysis in liver by α_1 -adrenergic agonists, vasopressin and angiotensin is mediated by Ca²⁺ acting on phosphorylase *b* kinase, which will then activate glycogen phosphorylase. Current evidence suggests a mobilization of Ca²⁺ from the endoplasmic reticulum triggered by inositol trisphosphate generated by hormone-initiated hydrolysis of phosphatidylinositol 4,5-bisphosphate in the plasma membrane [see Williamson *et al.* (1985) for a review]. Electrical stimulation of the sympathetic innervation of the liver also results in a cyclic AMP-independent glycogenolysis, which has been ascribed to the release of noradrenaline acting on α_1 -adrenoreceptors [see De Wulf & Carton (1981) for a review]. Since in a number of tissues ATP is a co-transmitter in the sympathetic nerve terminals (Burnstock, 1981), the question arises as to the possibility of a Ca²⁺-mediated purinergic control of liver glycogenolysis.

At least at high concentrations, exogenous ATP reproduces several effects of the Ca²⁺-dependent glycogenolytic hormones, such as glucose production at 0.1 mM (De Witt & Putney, 1983), Ca²⁺ redistribution at 0.3–0.5 mM (Krell *et al.*, 1983) and 0.8 mM (Bellomo *et al.*, 1984), phosphorylase activation at 0.8 mM (Bellomo *et al.*, 1984), hydrolysis of phosphatidylinositol 4,5-bisphosphate at 0.1 mM (Creba *et al.*, 1983) and increased inositol trisphosphate at 0.1 mM (Charest *et al.*, 1985). In the present paper, we show that ATP acts at much lower concentrations and present strong evidence for a common mode of action for ATP and the Ca²⁺-dependent glycogenolytic hormones.

EXPERIMENTAL

We used male Wistar-strain albino rats (200–250 g body wt.) that were fed *ad libitum*. Preparation, incubation and sampling of liver cells for the assay of phosphorylase *a* and the measurement of cyclic AMP were done as described by Keppens & De Wulf (1984). ATP, ADP, AMP and adenosine were from Sigma Chemical Co. The origin of the other chemicals has been given previously (Keppens & De Wulf, 1984).

RESULTS AND DISCUSSION

Fig. 1(a) shows the transient nature of phosphorylase activation after treatment of hepatocytes with different concentrations of ATP. Maximal activation is obtained within 15 s and is followed by a marked decline, most probably due to the rapid hydrolysis of the nucleotide by an ecto-ATPase of the cells (see Krell *et al.*, 1983). Indeed, a second addition of ATP causes identical activation patterns (results not shown). In contrast, the addition of adenosine results in a completely different time-dependent activation of phosphorylase (Fig. 1b), which is characterized by a lag period of about 10 s. ADP and AMP produce activation patterns similar to those of ATP and adenosine respectively (results not shown). We have not observed any interference with possible contaminants of commercial ATP preparations such as aluminium (Womack & Colowick, 1979) and vanadate (Cantley *et al.*, 1977).

Dose-response curves (Fig. 2) yield the following *K_a* values: 5×10^{-7} M for ATP, 2×10^{-6} M for ADP and about 5×10^{-5} M for AMP and adenosine. This order of potency indicates that ATP interacts with the P₂-purinergic receptors, according to the terminology of Burnstock (1981). This conclusion is confirmed by the lack of any glycogenolytic effect of ATP with cells depleted of Ca²⁺ by pretreatment with EGTA and by the absence of any effect on cyclic AMP concentrations up to 50 μ M-ATP (results not shown). The glycogenolytic ability of ATP is unlikely to be due to a kinase-initiated sequence of events, since the omission of Mg²⁺ from the incubation medium was without effect. ATP can thus be considered as being a cyclic AMP-independent Ca²⁺-dependent glycogenolytic agonist.

Additional evidence points to a common mode of action for ATP and the other Ca²⁺-dependent agonists. First, vasopressin and the α_1 -adrenergic agonists have been shown to induce a heterologous desensitization of the liver glycogenolytic response towards the Ca²⁺-dependent hormones, which implies that they all share a common mode of action (Bréant *et al.*, 1981). We show in Fig. 3 that vasopressin also decreases the glycogenolytic effect of ATP, with the same dose-dependency as its own glycogenolytic ability. Similar results have been obtained

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Fig. 1. Time-dependent activation of glycogen phosphorylase by ATP and adenosine

Liver cells were preincubated at 37 °C for 25 min with 10 mM-glucose and were then challenged with (a) ATP or (b) adenosine at the concentrations indicated. Phosphorylase *a* was assayed at the times indicated. This Figure shows results that are representative of several similar experiments.

Fig. 2. Dose-dependency of purinergic agonists for phosphorylase activation

Liver cells were challenged with increasing concentrations of ATP (○), ADP (●), AMP (△) or adenosine (▲). Phosphorylase *a* was assayed 20 s later. Values are given as means ± S.E.M. (where scale permits) for three experiments. Basal activity of phosphorylase *a* was 19.1 ± 3.5 munits/mg of protein.

Fig. 3. Dose-dependency of vasopressin to desensitize the glycogenolytic ability of ATP

Liver cells were incubated for 20 min with increasing concentrations of vasopressin. Phosphorylase *a* activities were then measured (△), and also 1 min after the addition of 10 μM-ATP (▲) or 10 nM-vasopressin (●). Values are given as means ± S.E.M. for three experiments.

Fig. 4. Effect of ATP on the glucagon-induced accumulation of cyclic AMP

Hepatocytes were incubated with 5 nM-glucagon (○); 10 μ M-ATP was added at 0 min (●) or 2 min (▲). Cyclic AMP concentrations were determined at the times indicated. Values are given as means $\pm$ S.E.M. for three experiments. Basal content of cyclic AMP was 3.4 ± 0.2 pmol/mg of protein.

with the α_1 -agonist phenylephrine as the desensitizing agent (results not shown) [owing to its rapid hydrolysis, ATP cannot be employed as a desensitizing agent, as we (Keppens & De Wulf, 1982) have shown that continuous presence of the agonist is mandatory to maintain the desensitized state]. These data strongly suggest that ATP uses the same effector system(s) as the Ca^{2+} -mediated hormones. Secondly, the Ca^{2+} -dependent hormones are able to decrease cyclic AMP in hepatocytes incubated with glucagon [see Keppens & De Wulf (1984) for references]. Fig. 4 illustrates this ability of ATP whether added together with glucagon or 2 min later. Thirdly, we (Keppens & De Wulf, 1984) have attributed this cyclic AMP-decreasing effect by angiotensin and vasopressin to an enhanced phosphodiesterase activity, since it is lost in the presence of several phosphodiesterase inhibitors. Similarly, in three independent experiments 3-isobutyl-1-methylxanthine (50 μ M) prevented 10 μ M-ATP from lowering the glucagon-induced cyclic AMP concentrations.

CONCLUSIONS

Two conclusions can be drawn from our data. First, liver seems to possess purinergic receptors of the P₂-subclass (terminology of Burnstock, 1981). On release of ATP by the sympathetic nerve terminals, these could, in conjunction with noradrenaline, initiate a Ca^{2+} -dependent glycogen breakdown. Interestingly, the K_a value for ATP (5×10^{-7} M; the present paper) compares favourably with that for noradrenaline (3×10^{-7} M; Bréant *et al.*, 1981). Secondly, ATP shares a common mode of action with the cyclic AMP-independent Ca^{2+} -dependent glycogenolytic hormones angiotensin, vasopressin and α_1 -agonists. This conclusion is substantiated by (1) their requirement for Ca^{2+} , (2) their inability to increase cyclic AMP, (3) their ability to undergo a heterologous desensitization by vasopressin and phenylephrine (an α_1 -agonist), (4) their ability to decrease cyclic AMP in hepatocytes challenged with glucagon, most probably mediated by an enhanced phosphodiesterase activity.

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