Guanine nucleotides can activate the insulin-stimulated phosphodiesterase in liver plasma membranes

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The insulin-stimulated cyclic AMP phosphodiesterase from liver plasma membranes is shown to be activated upon incubation with guanine nucleotides in the presence of ATP. The non-hydrolysable analogue of ATP, adenylyl imidodiphosphate failed to substitute for ATP in achieving activation. GTP, its non-hydrolysable analogues p[NH]ppG and GTP-γ-S, as well as GDP, all elicited activation. It is suggested that guanine nucleotides, and probably insulin, exert their effect on this enzyme through a distinct species of guanine nucleotide regulatory protein.

Cyclic AMP phosphodiesterase *Guanyl nucleotides* *Insulin* *Phosphorylation* *Guanyl nucleotide regulatory protein* *Liver plasma membranes*

1. INTRODUCTION

Many cellular functions are regulated by alterations in intracellular cyclic AMP concentrations [1]. In liver, the only mechanism of degrading cyclic AMP is through the action of cyclic AMP phosphodiesterases [2–4]. These enzymes exist in multiple forms within the liver, associated with both soluble and membrane-bound fractions [2–6]. In a variety of tissues the activity of certain species is affected by hormones (review [4]). In liver (review [4,7,8]) the activity of two distinct membrane-bound species can be modulated by glucagon and insulin in intact hepatocytes [7,8].

In purified liver plasma membranes, insulin can activate a high affinity peripheral cyclic AMP phosphodiesterase [9–13], which is bound to a specific, integral protein site on the plasma membrane [14]. This activation occurs at physiological concentrations of insulin [10] and is due to an insulin-triggered phosphorylation of the enzyme [10,13]. However, this enzyme can be activated by insulin in intact hepatocytes [7,8].

The activation of a number of hormones appears to be mediated or modulated by guanine nucleotide regulatory proteins [15,16]. Furthermore, a high affinity cyclic GMP phosphodiesterase in rod outer segments is light-activated through a process involving rhodopsin and a distinct guanine nucleotide regulatory protein [17–20].

Here, we show that guanine nucleotides elicit an activation of the peripheral, liver plasma membrane, cyclic AMP phosphodiesterase.

2. MATERIALS AND METHODS

Rat liver plasma membranes were prepared using a modification of the method in [21]. Male Sprague-Dawley rats (225–275 g body wt) were used. Typically, 3 rat livers were homogenized using 4 strokes of a Teflon–glass homogenizer taking 3 vol. liver with 1 vol. 1 mM $KHCO_3$ (pH 7.2). The homogenate was diluted to 120 ml with 1 mM $KHCO_3$ and subsequently centrifuged at 2000 *g* for 10 min. The resulting pellet was resuspended to ~80 ml with 1 mM $KHCO_3$ and added to 60 g sucrose. This sucrose was dissolved by stirring the homogenate for 30 min at 4°C. Plasma membranes were collected at the 42.5–48.2% (w/v)

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sucrose interface during centrifugation at 100 000 × *g* for 3 h in a swing out MSE prepsin 3 × 70 ml head. These membranes were removed from the density gradient and subsequently diluted with an equal volume of 1 mM $KHCO_3$. Membranes were finally collected by centrifugation at 25 000 × *g* for 10 min and resuspended in 1 mM $KHCO_3$ (pH 7.2) to 15–20 mg/ml prior to storage at −80°C.

The in vitro activation and assay of cAMP phosphodiesterase were essentially as [10]. Briefly prior to activation, membranes were diluted with 5 vol. 0.25 M sucrose containing 3 mM imidazole (pH 7.4) and collected by centrifugation at 15 000 × *g* for 10 min. This washing procedure was repeated once before the membranes were resuspended in 0.25 M sucrose containing 3 mM imidazole (pH 7.4). Aliquots of plasma membranes (500 μg protein) were incubated at 30°C for 5 min with an activation cocktail containing 10^{-4} M cyclic AMP, 4 mM ATP and 5 mM $MgCl_2$, 100 μM $CaCl_2$, 10^{-8} M insulin and 20 mM Tris–HCl (pH 7.4) final conc. All these solutions, including the Tris-buffer were made up freshly each day. After incubation the membranes were rapidly cooled and washed a further twice with 0.25 M sucrose containing 3 mM imidazole (pH 7.4). The high affinity peripheral cyclic AMP phosphodiesterase was solubilized with 0.4 M NaCl prior to assay with 4 × 10^{-7} M cyclic AMP at 30°C for 15 min as in [5].

Protein was determined by a modified microbiuret method [22]. Cyclic AMP, ATP, GTP, GTP-γ-S (guanosine-5′-*O*-[3-thiotriphosphate]) and pNHppG (guanine 5′-[β,γ-imidol] triphosphate) were purchased from Boehringer (Lewes, East Sussex). All other biochemicals including GDP (cat. no. G.6506) were from Sigma Chemical Co. (Surrey). Radiochemicals were obtained from Amersham International (Bucks). All other chemicals were of AR grade from BDH Chemicals (Poole, Dorset).

3. RESULTS AND DISCUSSION

Insulin can cause the phosphorylation and activation of a high affinity cyclic AMP phosphodiesterase associated with liver plasma membranes [10–13]. This enzyme is a peripheral protein [5], which appears to bind to a specific integral protein within the membrane [14]. As well as activating this phosphodiesterase in an isolated membrane preparation [10–13], insulin can also activate it in intact hepatocytes [7,8]. Under such conditions we also observed [7,8] a concomitant decrease in intracellular cyclic AMP concentrations. This suggests that the peripheral phosphodiesterase may have an important regulatory function within the cell. Although activation of this enzyme proceeds via a phosphorylation mechanism [13] it is, at present, unclear as to how insulin triggers this event. However, the enzyme might bind to the insulin receptor itself [14]. Occupancy of the insulin receptor, by hormone, could then result in a conformational change in the associated phosphodiesterase, allowing it to become a substrate for phosphorylation.

We show here that guanine nucleotides elicit the activation of the peripheral cyclic AMP phosphodiesterase from liver plasma membranes

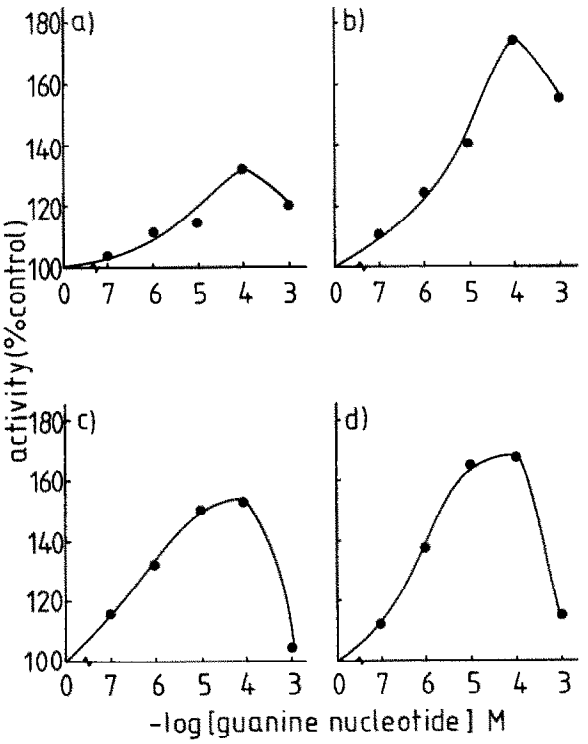

Fig. 1. Guanine nucleotide activation of plasma membrane phosphodiesterase. Activations and assay of the peripheral cyclic AMP phosphodiesterase were performed as in section 2. Results are the average of 3 expt. Guanine nucleotides used were: (a) GTP; (b) GDP; (c) p[NH]ppG; (d) GTP-γ-S.

Table 1

Effect of guanine nucleotides on the activation of the peripheral, liver plasma membrane phosphodiesterase (activity, % of control)

Ligand	Guanine nucleotide, final conc.	
	10^{-6} M	10^{-4} M
GTP	115 ± 4	136 ± 6
GTP + insulin	131 ± 8	156 ± 5
GDP	125 ± 5	172 ± 3
GDP + insulin	120 ± 6	144 ± 5
GTP-γ-S	140 ± 6	157 ± 5
GTP-γ-S + insulin	168 ± 4	190 ± 4
p[NH]ppG	141 ± 4	157 ± 3
p[NH]ppG + insulin	182 ± 10	229 ± 4

The results are the average of 3 expt. done using a single membrane preparation (errors are ± SD). This typifies the results that were obtained using 3 different membrane preparations in all. In this instance insulin elicited at 130 ± 5% activation of the peripheral plasma membrane phosphodiesterase. Activations and phosphodiesterase assay were done as in section 2

(fig. 1, table 1). This process is presumably mediated through the phosphorylation of the enzyme as adenylyl imidodiphosphate, a non-hydrolysable analogue of ATP, failed to substitute for ATP in achieving activation. Furthermore, GTP does not appear to act as the phosphate donor as p[NH]ppG and GTP-γ-S both caused the activation of this enzyme when ATP was present. In each case the degree of activation elicited by the guanine nucleotides was dose dependent, although mM levels of these nucleotides, especially the non-hydrolysable analogues tended to block the response (fig. 1).

Interestingly, GDP was quite as effective as GTP, if not more so, in activating the enzyme (table 1). However, there was no observable effect of insulin in activating the enzyme when GDP was present. This contrasts with a small effect seen with GTP and near additive or synergistic effects observed using the two non-hydrolysable GTP analogues, p[NH]ppG and GTP-γ-S (table 1).

These observations bear analogy with the light activation of the high affinity cyclic GMP phosphodiesterase in rod outer segments [17–20]. It is also a peripheral protein which associates with the membrane through binding to integral protein(s) rather than any lipid interaction [17]. Here, the photo-excitation of rhodopsin elicits the activation of this enzyme in a guanyl nucleotide-mediated process [17–20]. The mechanism of this activation is unknown, no evidence having been provided to suggest that a phosphorylation step is involved [17,23,24]. However, rhodopsin does trigger the light-activated phosphorylation of a number of proteins [23–26], an effect which can be enhanced by GTP [24].

The peripheral, liver plasma membrane phosphodiesterase could also be activated through a process involving a guanine nucleotide regulatory protein. Indeed, treatment of hepatocytes with just cholera toxin, which is known to specifically modify guanine nucleotide regulatory proteins [16], activates this enzyme (unpublished). Cholera toxin has also been shown to elicit the activation of the retinal enzyme in the absence of light [27]. The K_a for GTP-activation of the retinal phosphodiesterase [19] is very similar to that we observe for the liver enzyme (3–5 μM, range, n = 3). Furthermore, in both systems ([19] fig. 1), the K_a for activation by the non-hydrolysable analogues of GTP (0.5–1 μM, range n = 3) are considerably lower than for GTP itself. However, the retinal enzyme may be activated by a guanine nucleotide regulatory protein that expresses GTPase activity [17–20]. Thus it is assumed [17–20,28] that GDP would not activate the enzyme. This is in contrast with the liver phosphodiesterase activated by GDP (table 1, fig. 1). However, in liver, guanine nucleotide regulatory protein(s) acting on adenylate cyclase can be activated by GDP under conditions where no transphosphorylation exists [29,30]. We see here (fig. 1) that GDP is a more effective activator of the phosphodiesterase than GTP. Thus the binding of guanine nucleotides themselves might elicit changes in the functioning of the regulatory protein, rather than it being achieved by GTP hydrolysis.

In liver plasma membranes [15,16], glucagon exerts its activating effect on adenylate cyclase through a stimulatory guanine nucleotide regulatory protein (N_s). This protein (N_s) is unlikely to be a candidate for action on the phosphodiesterase as glucagon does not activate the phosphodiesterase in either broken membranes [10] or in intact cells [7,8]. Indeed the K_a for GTP,

expressed by glucagon-stimulated adenylate cyclase is of the order of 0.05–0.1 μM [31–33]. This is very different from the K_a-values of 3–5 μM we observe for GTP/GDP activation of the phosphodiesterase. However, as well as the so-called N_s regulatory proteins, a family of distinct guanine nucleotide regulatory proteins (N_i) have been identified based upon their ability to mediate inhibitory responses on adenylate cyclase [15]. These proteins (N_i) have an affinity for GTP which is around two orders of magnitude lower than that exhibited by N_s [15,34,35]. Furthermore, this species appears to exhibit a considerably higher affinity for the non-hydrolysable analogues of GTP than for GTP itself [36,37], which is in accord with our observations (fig. 1) of their ability to activate the liver phosphodiesterase. As multiple species of guanine nucleotide regulatory proteins appear to exist in liver plasma membranes [15] it is possible that the activation of the phosphodiesterase we observe is mediated by a guanine nucleotide protein with the characteristics of N_i.

It remains to be seen whether insulin's activation of the peripheral phosphodiesterase is also mediated via a guanine nucleotide regulatory protein. Certainly one can draw an analogy with the light activation of retinal phosphodiesterase, where the involvement of such a regulatory protein has been clearly demonstrated [17–20]. In sarcolemma membranes [38], insulin causes the ATP-dependent phosphorylation of membrane proteins through a GTP-requiring process. We have demonstrated ([7,8], unpublished) that in intact hepatocytes the ability of insulin to activate the peripheral phosphodiesterase is either severely reduced or blocked by treatments which modify guanine nucleotide regulatory proteins. Therefore, insulin may exert certain of its cellular effects through guanine nucleotide regulatory proteins.

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