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Inhibition by Glucagon of the cGMP-inhibited Low-K_m cAMP Phosphodiesterase in Heart Is Mediated by a Pertussis Toxin-sensitive G-protein*

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We have recently reported that glucagon activated the L-type Ca^{2+} channel current in frog ventricular myocytes and showed that this was linked to the inhibition of a membrane-bound low-K_m cAMP phosphodiesterase (PDE) (Méry, P. F., Brechler, V., Pavoine, C., Pecker, F., and Fischmeister, R. (1990) *Nature* 345, 158–161). We show here that the inhibition of membrane-bound PDE activity by glucagon depends on guanine nucleotides, a reproducible inhibition of 40% being obtained with 0.1 μM glucagon in the presence of 10 μM GTP, with GTP > GTPγS, while GDP and ATPγS were without effect. Glucagon had no effect on the cytosolic low-K_m cAMP PDE, assayed with or without 10 μM GTP. Glucagon inhibition of membrane-bound PDE activity was not affected by pretreatment of the ventricle particulate fraction with cholera toxin. However, it was abolished after pertussis toxin pretreatment. Mastoparan, a wasp venom peptide known to activate G_i/G_o proteins directly, mimicked the effect of glucagon.

PDE inhibition by glucagon was additive with the inhibition induced by Ro 20-1724, but was prevented by milrinone. This was correlated with an increase by glucagon of cAMP levels in frog ventricular cells which was not additive with the increase in cAMP due to milrinone. We conclude that glucagon specifically inhibits the cGMP-inhibited, milrinone-sensitive PDE (CGI-PDE).

Insensitivity of adenylylcyclase to glucagon and inhibition by the peptide of a low-K_m cAMP PDE were not restricted to frog heart, but also occurred in mouse and guinea pig heart.

These results confirm that two mechanisms mediate the action of glucagon in heart: one is the activation of adenylylcyclase through G_s, and the other relies on the inhibition of the membrane-bound low-K_m CGI-PDE, via a pertussis toxin-sensitive G-protein.

Both adenylylcyclase and cyclic nucleotide phosphodiesterases (PDE)[1] control the cellular level of cAMP. The hormonal regulation of the cardiac adenylylcyclase, mediated by G_s and G_i proteins, has been described extensively (1, 2). In contrast, the mechanisms of the hormonal regulation of PDE activity are still debated, probably due to the existence of numerous PDE isozymes whose identification and distribution remain unclear (3–5).

Glucagon is known to exert a positive inotropic effect on heart contraction, and recently, we found that it activated the L-type Ca^{2+} channel current in rat and frog ventricle (6). While in rat ventricle, this effect was correlated with adenylylcyclase stimulation, in frog ventricle the effect was linked to the inhibition of a low-K_m cAMP PDE (6). This observation further emphasised the role of PDEs in the control of cardiac contraction (7–9), but raised the question as to the isozyme family of the hormone-sensitive PDE and the mechanism involved.

In heart, four families of PDEs have been defined: 1) the Ca^{2+}-calmodulin-regulated PDEs that hydrolyze both cAMP and cGMP; 2) a cGMP-stimulated PDE that hydrolyzes cAMP in a relatively high range of concentrations (10–100 μM); 3) the cAMP-specific PDE which has a high selectivity for cAMP as substrate (K_m < 1 μM) and is inhibited by rolipram and Ro 20-1724; 4) the cGMP-inhibited PDE (CGI-PDE) which has a high affinity for cAMP (K_m < 1 μM) and is inhibited by low concentrations of cGMP and bipyridine PDE inhibitors such as milrinone (4). How these different types of PDEs contribute to the regulation of cAMP levels remains unclear, and it is likely that the intracellular distribution of phosphodiesterase isozymes is an important factor in determining their regulatory role (8). It is worth noting that in the cardiac cell 50–70% of the total PDE activity, while only 10–20% in smooth muscle, kidney, or platelets, is associated with the membranes (5).

In the present study, we have measured the phosphodiesterase activity in the particulate and cytosolic fractions of frog heart ventricle with the aim of characterizing the process of inhibition and identifying the isozyme family of the low-K_m PDE inhibited by glucagon. We have examined the role of guanine nucleotides, the effect of bacterial toxins, cholera toxin, and pertussis toxin, and the additivity of the effect of glucagon with that of the specific inhibitors of each low-K_m PDE subtype, Ro 20-1724 and milrinone, respectively. We show that glucagon specifically inhibits the membrane-bound CGI-PDE, and that this effect is mediated by a pertussis toxin-sensitive G-protein.

MATERIALS AND METHODS

Preparation of Heart Ventricle Particulate and Cytosolic Fractions—All preparative procedures were carried out at 4 °C. Hearts were removed from two frogs (*Rana esculenta*) and placed in 10 ml of

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[1] The abbreviations used are: PDE, phosphodiesterase; CGI-PDE, cGMP-inhibited, milrinone-sensitive PDE; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); ATPγS, adenosine 5′-*O*-(3-thiotriphosphate); IAP, pertussis toxin.

50 mM Tris-HCl, pH 7.5, containing 10 mM $MgCl_2$, 1 mM EDTA. Ventricles freed of blood vessels and connective tissue were then placed in 2 ml of fresh buffer, lacerated with scissors, and homogenized with a glass-Teflon homogenizer. The homogenate was centrifuged at 14,000 × *g* for 3 min. The supernatant and the pellet resuspended in buffer at 2–3 mg of protein/ml were stored in liquid nitrogen until use. The particulate fractions from mouse and guinea pig heart ventricles were prepared according to the same procedure.

Treatment of Frog Ventricle Particulate Fractions with Toxins—Prior to use, cholera and pertussis toxins were activated by incubation with 20 mM dithiothreitol for 30 min at 37 °C. Frog ventricle particulate fraction (3 mg of protein) was incubated for 30 min at 30 °C with either 300 μg/ml activated cholera toxin, or 10 μg/ml activated pertussis toxin, or vehicle, in a final volume of 1.5 ml containing 20 mM Hepes, pH 7.4, 160 mM NaCl, 1 mM ATP, 3 mM dithiothreitol, 0.1 mM GTP, and 10 μM NAD. Samples were then centrifuged at 15,000 × *g* for 5 min and washed twice by dilution in 3 ml final of ice-cold 50 mM Tris-HCl, pH 7.5, containing 10 mM $MgCl_2$, 1 mM EDTA. The final pellet was resuspended in the same buffer and was used for phosphodiesterase activity determination.

Low-K_m cAMP Phosphodiesterase (PDE) Assay—PDE activity in frog ventricle particulate fraction has been characterized previously (6) and was determined according to the two-step assay procedure described in Ref. 10. The assay medium contained: 20 mM Hepes, pH 7.6, 120 mM CsCl, 5 mM EGTA, 4 mM $MgCl_2$, 2 μM [^{3}H]cAMP (10^5 cpm), in a final volume of 0.4 ml. Incubation was initiated by the addition of 40–50 μg of protein, lasted for 10 min at 30 °C, and was terminated by a 45-s ebullition. Tubes were then cooled at 4 °C, and a second incubation step was run for 10 min at 30 °C, initiated by the addition of 0.1 ml of 5′-nucleotidase of cobra venom (at a final concentration of 1 mg/ml) in order to convert the 5′-[^{3}H]AMP formed into [^{3}H]adenosine. Tubes were then cooled at 4 °C and diluted with 1 ml of ice-cold methanol. Assay mixtures were applied to columns filled with 3 ml of Dowex AG1-X2 (Bio-Rad, chloride form, 200–400 mesh) diluted 1:4 in methanol. Before use, anion exchange resin had been washed successively with 0.5 N HCl, H_2O, 0.5 N NaOH, H_2O, 0.5 N HCl, and then repeatedly with H_2O until pH 5 was reached, as described in Ref. 10. After elution with 1 ml of methanol, assays were diluted with 10 ml of Beckman Ready Protein and counted in a scintillation counter. Blank values were estimated by assays boiled immediately after the addition of proteins and were subtracted from all data points. Results were obtained from triplicate determinations.

Guanylylcyclase Assay—For control experiments, guanylylcyclase activity was assayed as described in Ref. 11 and in conditions similar to those used for the measurement of PDE activity. The assay medium contained, in a final volume of 80 μl: 20 mM Hepes, pH 7.6, 120 mM CsCl, 5 mM EGTA, 4 mM $MgCl_2$, 2 μM cAMP, 1 mM [^{3}H]cGMP (3 × 10^4 cpm), 1 mM [^{32}P]GTP, and a regenerating system consisting of 25 mM creatine phosphate and 1 mg/ml phosphocreatine kinase. The incubation was initiated by the addition of 40–50 μg of protein of ventricle particulate fraction and lasted for 10 min at 30 °C.

Adenylylcyclase Assay—Adenylylcyclase was assayed as described previously (6), with 50 μM GTP added to the assay medium. The incubation was initiated by the addition of 40–50 μg of protein of ventricle particulate fraction and lasted for 10 min at 30 °C.

Determination of cAMP Content—Ventricular cells were dispersed from frog hearts as described in Refs. 6 and 12. After perfusion with the dissociation medium, the heart was placed in 10 ml of Ringer solution containing: 10 mM Hepes, pH 7.4, 107 mM NaCl, 20 mM CsCl, 4 mM $NaHCO_3$, 0.8 mM NaH_2PO_4, 5 mM glucose, 5 mM sodium pyruvate. Atrium and bulbus arteriosis were discarded. The ventricle was lacerated gently with forceps and stirred. The cell suspension was then filtered prior to storage at 4 °C for 1–24 h.

Drug incubations were performed at room temperature; 500-μl aliquots of cell suspension (100 × 10^3–200 × 10^3 cells/ml) were placed in hemolysis tubes, and drugs were added as indicated in the legend to Fig. 6. The incubation was stopped after 15 min by the addition of 0.5 N perchloric acid. Tubes were vortexed, placed at 4 °C for 5 min, and centrifuged at 10,000 × *g* for 5 min. The supernatants were then buffered to pH 7 with 2.5 M $KHCO_3$ solution and centrifuged again. The cAMP content of each supernatant was determined by radioimmunoassay. Cellular concentration of cAMP was calculated assuming that the volume of a frog ventricular myocyte is 5 pl (13). Proteins were determined using the Bio-Rad assay with bovine serum albumin as standard.

Nucleotides, cobra venom, cholera toxin, and mastoparan were purchased from Sigma. Pertussis toxin was from List Biological (Campbell, CA), crystallized porcine glucagon from Novo (Denmark), and [^{3}H]cGMP, [^{32}P]GTP, and [^{3}H]cAMP were from Amersham. Milrinone (WIN 47 203) was obtained from Sterling-Winthrop (New York) and Ro 20–1724 from Roche. Radioimmunoassay kit for cAMP was obtained from Institut Pasteur Production (Marnes la Coquette, France).

RESULTS

Influence of GTP on Low-K_m cAMP PDE Inhibition by Glucagon in Frog Ventricle Particulate Fraction—As previously reported (6), glucagon reduced the low-K_m cAMP PDE activity in frog ventricle particulate fraction (Fig. 1). However, substantial variations in the degree of inhibition induced by glucagon were observed among different particulate preparations. As shown in Fig. 1, these variations could be overcome if micromolar concentrations of GTP were added to the assay medium, and, as summarized in Fig. 2, a highly reproducible 40% maximal PDE inhibition was observed with 1 μM glucagon in the presence of 10 μM GTP. The half-maximal effect of glucagon occurred at 20 nM (Fig. 2).

When added alone, GTP or GTPγS had no effect on the basal PDE activity (Table I). However, both nucleotides potentiated (or revealed) the effect of glucagon. GTPγS was less efficient than GTP since only 24% maximal inhibition of PDE activity was reached with 1 μM glucagon in the presence of 10 μM GTPγS (Table I). GDP or ATPγS, added with or

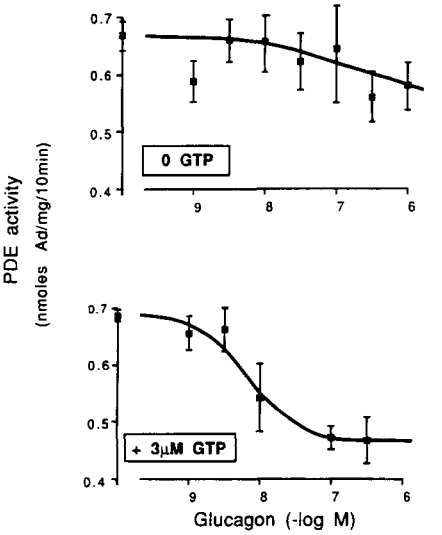

FIG. 1. **Low-K_m cAMP PDE inhibition by glucagon in frog ventricle particulate fraction depends on GTP.** PDE activity was measured as described under "Materials and Methods" with varying concentrations of glucagon in the absence (*top*) or in the presence (*bottom*) of 3 μM GTP. Results are from a typical experiment and are the mean ± S.E. of triplicate determinations.

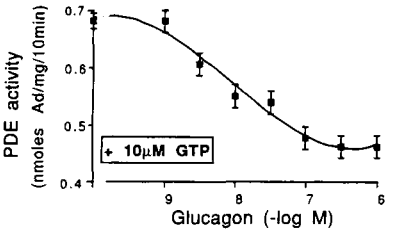

FIG. 2. **At 10 μM GTP, maximal inhibition by glucagon of the low-K_m cAMP PDE was observed in all preparations of frog ventricle particulate fraction.** PDE activity was measured as described under "Materials and Methods," in particulate fractions obtained from nine different preparations, with varying concentrations of glucagon and in the presence of 10 μM GTP. Results are the mean ± S.E. of 27 determinations obtained from 9 experiments.

TABLE I

Effect of glucagon in the presence of different nucleotides and effect of mastoparan on the low-K_m cAMP PDE activity in frog ventricle particulate fraction

PDE activity was measured as described under "Materials and Methods" either in the presence of 10 μM GTP, GTPγS, GDP, or ATPγS with or without 1 μM glucagon or in the presence of different concentrations of mastoparan. Results are the mean ± S.E. of three to nine determinations.

Compound	PDE activity		
	Without glucagon	With 1 μM glucagon	Inhibition
	nmol adenosine/mg/10 min		%
None	0.71 ± 0.02		
10 μM GTP ($n = 9$)	0.68 ± 0.03	0.46 ± 0.02	35
10 μM GTPγS ($n = 4$)	0.69 ± 0.03	0.54 ± 0.03	24
10 μM GDP ($n = 4$)	0.68 ± 0.02	0.69 ± 0.02	
10 μM ATPγS ($n = 6$)	0.72 ± 0.04	0.72 ± 0.04	
Mastoparan ($n = 3$)			
1 μM	0.70 ± 0.02		
5 μM	0.60 ± 0.03		14
50 μM	0.48 ± 0.01		32

without glucagon, had no effect on PDE activity (Table I).

Effects of Cholera Toxin and Pertussis Toxin on the Inhibition of PDE by Glucagon—Bacterial toxins have proved to be useful tools for the identification of distinct G-proteins. In heart, pertussis toxin (IAP) ADP-ribosylates the α subunits of G_i and G_o, leading to their blockade in the trimeric form and their inhibition, while cholera toxin ADP-ribosylates the α subunits of G_s proteins, leading to their dissociation from βγ and to their persistent activation (for reviews, see Refs. 1, 14, and 15).

We examined the consequences of *in vitro* treatments of ventricle particulate fraction with cholera or pertussis toxins on the sensitivity of PDE to glucagon. Cholera toxin treatment affected neither basal PDE activity nor the ability of glucagon to inhibit PDE activity (Fig. 3, *top*). In contrast, IAP treatment of the particulate fraction totally abolished inhibition of PDE by glucagon, without changing the basal PDE activity (Fig. 3, *bottom*). These results suggest that a pertussis toxin-sensitive G-protein controls the inhibition of PDE by glucagon. These results also imply that the action of glucagon is mediated by membrane receptors. This hypothesis was further supported by the absence of sensitivity to glucagon of the low-K_m cAMP PDE in the cytosolic fraction, whether the assay was carried in the presence or absence of 10 μM GTP (Fig. 4).

Effect of Mastoparan—Mastoparan is a peptide isolated from wasp venom which possesses a variety of biological effects. It has been shown to activate G-proteins (16) by interacting with their carboxyl (17) and amino (18) termini, mimicking agonist-liganded receptors. We have examined the effect of mastoparan on PDE activity. As shown in Table I, mastoparan mimicked the effect of glucagon. The peptide evoked a dose-dependent inhibition of PDE activity, leading to a 30–35% maximal inhibition at a concentration of 50 μM, *i.e* in the range of the concentrations needed to activate G_i and G_o (16). The inhibitory effect of mastoparan was not observed after pertussis toxin treatment of the ventricle particulate fraction (not shown). This reinforces the proposal that G_i/G_o proteins may be coupled to membrane-bound low-K_m cAMP PDE(s).

Characterization of the Low-K_m cAMP PDE Isozyme Sensitive to Glucagon Inhibition—Two isozyme families of low-K_m cAMP PDEs have been identified in heart on the basis of their sensitivity to cGMP and synthetic inhibitors. One isozyme family is inhibited by low concentrations of cGMP and cardiotonic bipyridine inhibitors such as milrinone (19). The other isozyme family is not regulated by cGMP and is inhibited by the antidepressant Ro 20-1724 (3). As shown in Fig. 5, both milrinone and Ro 20-1724 caused a 30–35% inhibition of basal PDE activity in frog particulate fraction, comparable to that induced by glucagon. This indicated the presence of the two low-K_m cAMP PDE isozyme families in frog ventricular particulate fraction and confirmed electrophysiological data in intact cells (8). A simple way to characterize which of the PDE isozymes was sensitive to glucagon was to examine

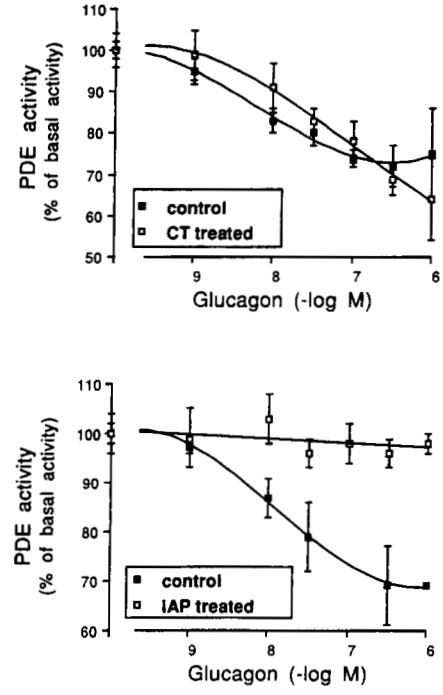

FIG. 3. **Consequences of *in vitro* treatment of frog ventricle particulate fraction with cholera toxin or pertussis toxin on low-K_m cAMP PDE inhibition by glucagon.** Frog ventricle particulate fractions were subjected to treatment with cholera toxin (*CT treated*) or pertussis toxin (*IAP treated*) or vehicle (control) as described under "Materials and Methods." PDE activity was measured as described under "Materials and Methods" with varying concentrations of glucagon in the presence of 10 μM GTP. Results are the mean ± S.E. of three experiments and are expressed as the percentage of basal PDE activity determined in the presence of 10 μM GTP (*CT treated*: 0.33 ± 0.05 nmol of adenosine/mg/10 min; *control CT*: 0.32 ± 0.04 nmol of adenosine/mg/10 min; *IAP treated*: 0.35 ± 0.01 nmol of adenosine/mg/10 min; *control IAP*: 0.36 ± 0.01 nmol of adenosine/mg/10 min).

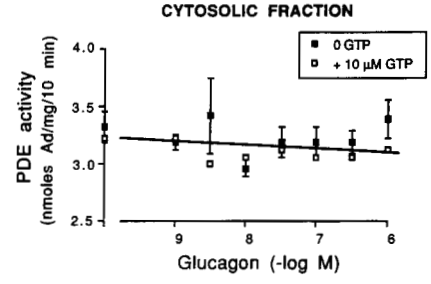

FIG. 4. **Absence of effect of glucagon on the cytosolic low-K_m cAMP PDE.** The cytosolic fraction was prepared as described under "Materials and Methods," and the cytosolic low-K_m cAMP PDE activity (40 μg of protein/assay) was measured as described under "Materials and Methods" with varying concentrations of glucagon in the absence or in the presence of 10 μM GTP. Results are the mean ± S.E. of three experiments.

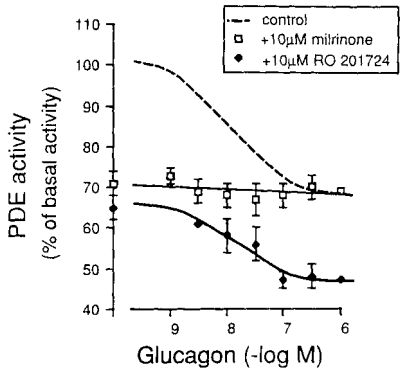

FIG. 5. **The inhibitory effect of glucagon on the low-K_m cAMP PDE in frog ventricle particulate fraction is additive with the effect of Ro 20-1724, but not with the effect of milrinone.** PDE activity was measured as described under "Materials and Methods" with varying concentrations of glucagon, in the presence of 10 μM GTP, and with milrinone or Ro 20-1724 added to the assay medium at a final concentration of 10 μM. Results are the mean ± S.E. of three to six experiments and are expressed as a percentage of basal activity determined in the presence of 10 μM GTP (0.68 ± 0.01 nmol of adenosine/mg/10 min). - - -, control taken from Fig. 2.

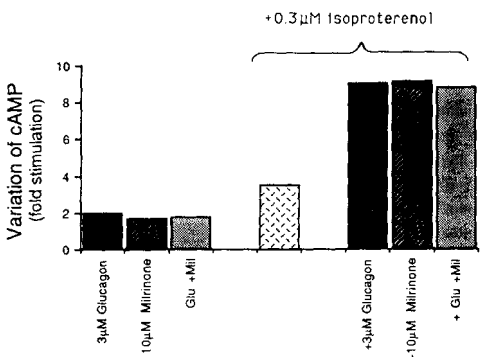

FIG. 6. **Absence of additive effects of glucagon and milrinone on cAMP level in frog ventricular cells.** Ventricular cells were isolated, incubated with glucagon, milrinone, and isoproterenol, and cyclic AMP content was determined as described under "Materials and Methods." Drugs were successively added to a final volume of 500 μl as follows: at time $t = 0$, 5 μl of 30 μM isoproterenol; at $t =$ 5 min, 5 μl of 1 mM milrinone; at $t = 10$ min, 5 μl of 300 μM glucagon. An equivalent volume of Ringer's solution was added to control tubes. In control conditions, cAMP concentration was 4.7 ± 2.3 μM. Results are the mean of six experiments.

whether the effect of glucagon was additive with that of milrinone or Ro 20-1724. The addition of increasing concentrations of glucagon evoked a dose-dependent inhibition of PDE activity in the presence of Ro 20-1724 (Fig. 5). In contrast, in the presence of milrinone, glucagon failed to evoke any further inhibition of PDE activity (Fig. 5). That the effects of glucagon and milrinone on PDE activity were not additive was also illustrated by measuring cAMP levels in intact frog ventricular cells. A 5-min application of 3 μM glucagon or 10 μM milrinone produced a 2-fold increase in cAMP level over the basal level (Fig. 6), and, as expected, potentiated the elevation in cAMP level produced by 0.3 μM isoproterenol through adenylylcyclase activation. Increases in cAMP induced by glucagon and milrinone were not additive, in the presence, or in the absence, of isoproterenol (Fig. 6). These results strongly suggest that the milrinone-sensitive CGI-PDE is the target of glucagon action in frog ventricle.

We had to consider the possibility that inhibition of the CGI-PDE by glucagon might be secondary to the activation by glucagon of a membrane-associated guanylylcyclase. We

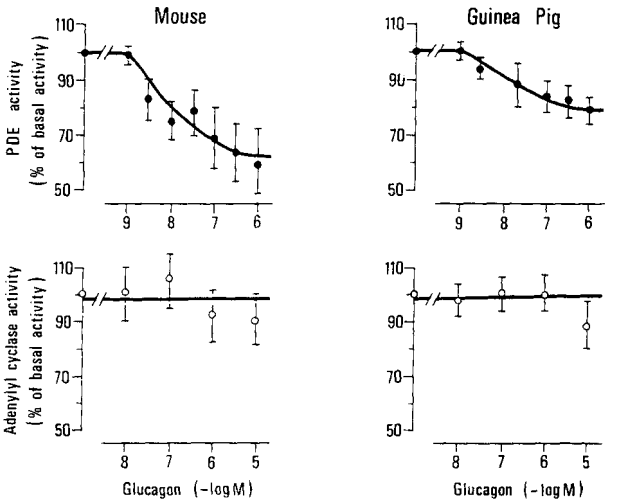

FIG. 7. **Inhibition by glucagon of the low-K_m cAMP PDE in particulate fractions obtained from mouse and guinea pig heart ventricles.** Particulate fractions were prepared from mouse and guinea pig heart ventricles according to the procedure described for frog heart ventricle. Low-K_m cAMP PDE and adenylylcyclase activity were measured as described under "Materials and Methods" with varying concentrations of glucagon and in the presence of 10 μM or 50 μM GTP, respectively. Results are the mean ± S.E. of nine determinations obtained from three experiments. Basal low-K_m cAMP PDE activity was 0.73 ± 0.10 and 2.0 ± 0.1 nmol of adenosine/mg/10 min in mouse and guinea pig ventricular particulate fractions, respectively. Basal adenylylcyclase activity was 0.97 ± 0.11 and 3.30 ± 0.25 nmol of cAMP/mg/10 min, respectively.

therefore assayed guanylylcyclase activity in ventricle particulate fraction in the conditions described under "Materials and Methods" similar to those used for the measurement of PDE activity, as a function of glucagon concentrations ranging from 0.1 nM to 1 μM. We did not observe any effect of glucagon on the guanylylcyclase activity in the ventricle particulate fraction (not shown).

Sensitivity to Glucagon of the Low-K_m cAMP PDE in Mouse and Guinea Pig Heart Ventricle—The inability of glucagon to stimulate adenylylcyclase in mouse (20) and in guinea pig heart ventricle (21) has been reported previously in the literature and led us to examine the sensitivity of the low-K_m cAMP PDE in heart ventricle of both species. As shown in Fig. 7, we confirmed the absence of responsiveness to glucagon of adenylylcyclase in mouse as well as in guinea pig ventricle. In both ventricle particulate fractions, we also observed a dose-dependent inhibition by glucagon of the low-K_m cAMP PDE (Fig. 7). Maximal inhibitions of 35 and 25% of the low-K_m cAMP PDE in mouse and guinea pig ventricle, respectively, were observed with 1 μM glucagon.

DISCUSSION

In the present study, we show that the membrane-bound CGI-PDE in frog heart is inhibited by glucagon through the activation of a pertussis toxin sensitive G-protein.

Hormonal regulation of PDEs has already been reported in various cell types, but several different mechanisms have been implicated, which are reviewed in Ref. 22 and are summarized below. As a first example, Ca^{2+}-calmodulin-sensitive PDE activation by muscarinic agonists has been demonstrated in canine thyroid (23) and in human astrocytoma cells (24, 25). In the latter case, the effect was lost in the absence of extracellular calcium or upon cell lysis, and it was proposed that Ca^{2+}-dependent PDE activation could be subsequent to inositol phosphate-mediated mobilization of cellular Ca^{2+} (25, 26).

As a second example, the long-term cAMP-dependent induction of the low-K_m cAMP PDE, insensitive to cyclic GMP and selectively inhibited by rolipram, has been described in various cell types (27 and for a review see Ref. 22).

It has also been shown that agents that increase intracellular cAMP produce a rapid increase in the membrane-bound CGI-PDE activity, thus glucagon in rat liver (28, 29), the adrenergic agonist isoproterenol in rat adipocytes (30, 31), and prostaglandins in human platelets (32). The cAMP-dependent activation of the CGI-PDE has been characterized further in rat adipocytes and human platelets, and it was found that the same enzyme is under the control of insulin, via a protein kinase C-dependent phosphorylation process at a site most likely different from the serine which is phosphorylated by the cAMP-dependent protein kinase (30–32). It should be mentioned that, in hepatocytes, insulin also stimulates the tyrosyl phosphorylation and activation of a membrane-bound, rolipram-sensitive cAMP PDE (33).

Finally, Buxton and Brunton have described PDE activation by α_1-adrenergic agonists in rat ventricular myocytes (34, 35). However, neither the mechanism nor the location of the enzyme in membrane or in cytosol was defined.

Inhibition of the membrane-bound CGI-PDE in frog ventricle by glucagon is the first evidence for hormonal inhibition and the existence of physiological inhibitors of low K_m cAMP PDE. The inhibition of the CGI-PDE by glucagon cannot be related to cAMP-dependent phosphorylation, since glucagon does not activate adenylylcyclase in frog heart (6). Furthermore, our *in vitro* assay conditions did not favor phosphorylation since no ATP was included.

Receptors were first thought to be highly selective for individual G-proteins. However, it is now apparent that a signal can be routed from one receptor to multiple G-proteins. Thus, the β-adrenergic receptor, which usually regulates G_s, can also interact with G_i, and perhaps with other G-proteins (36, 37). The coupling of glucagon receptors to G_s, which mediates adenylylcyclase activation, is commonly known. Their possible coupling to G_i could be supposed due to the inhibition of adenylylcyclase by glucagon reported in rabbit heart (38). But this was seen only in the presence of a high guanine nucleotide concentration (38), and the coupling of glucagon receptors to a pertussis toxin-sensitive G-protein under physiological conditions, reported herein, is a new observation. Taken together, these data suggest that the nature of the responses to receptor activation depend not only on the specific properties of the receptor molecule, but also on the cell type in which the receptor is expressed. It may be questioned whether the relative amounts of G-proteins are modulated by the hormonal status or pathological states, and whether this may determine the coupling to specific receptors.

The other example of a role for a G-protein in the regulation of phosphodiesterase activity is given by transducin which couples the light-activated receptor, rhodopsin, to the cGMP-PDE in retinal outer segment (39).

It appears that the membrane-bound CGI-PDE plays a critical role for hormonal action in various cell types. In fact, the singular role of the CGI-PDE in the regulation of cardiac contractility, as compared to the other PDE subtypes, is obvious from the potent cardiotonic action of its specific inhibitors (40). This would imply that this enzyme regulates a specific pool(s) of cAMP, and that the intracellular distribution of phosphodiesterase isozymes is an important factor in determining their regulatory function. Accordingly, it has been proposed that the inotropic efficacy of CGI-PDE inhibitors may depend upon the association of the enzyme with the sarcoplasmic reticulum and is diminished in animal species whose CGI-PDE activity is predominantly cytoplasmic (41). Hormonal inhibition of the CGI-PDE mediated through a G-protein, as reported here, is associated with an activation of Ca^{2+} current, further strengthening the potential role of the membrane-bound form of the enzyme in the control of cardiac contraction. Several studies also give evidence for the compartmentalization of cAMP in the cardiac cell (3, 42, 43).

As reported in previous studies, glucagon may act as a prohormone and be processed in its target tissues, in rat liver (44) and chick embryo heart ventricle (45), into the fragment (19–29), referred as to mini-glucagon, which has its own biological activity (45–47). It should be pointed out that we found no effect of mini-glucagon on either adenylylcyclase or phosphodiesterase activity, and that the peptide did not antagonize the activation or inhibition by glucagon of adenylylcyclase or phosphodiesterase, respectively. In conclusion, it has long been thought that adenylylcyclase, through the activation of a G_s protein, was the unique effector in the action of glucagon. We show here that PDE inhibition, mediated by a G_i/G_o protein, is a novel pathway of glucagon action which also leads to an increase in cellular cyclic AMP. It should be pointed out that this phenomenon would not be restricted to amphibians since we also report the inhibition by glucagon of a low K_m-cAMP PDE in mouse and guinea pig heart ventricle.

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