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Hormonally specific expression of cardiac protein kinase activity

(cardiac cyclic AMP-dependent protein kinase/hormonal specificity/glycogenolytic cascade/prostaglandin E_1/ β-adrenergic stimulation)

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ABSTRACT **The relationship between the effects of isoproterenol and prostaglandin E_1 (PGE_1) on contractile state, cyclic AMP accumulation, and the activation states of protein kinase (ATP: protein phosphotransferase, EC 2.7.1.37), phosphorylase kinase, glycogen synthase, and glycogen phosphorylase have been studied in the isolated perfused rat heart. Perfusion of hearts with isoproterenol (10 or 80 nM) caused enhancement of left ventricular *dP/dt* (*P*, pressure), increased intracellular cyclic AMP, increased the activation states of protein kinase, phosphorylase kinase, glycogen phosphorylase, and conversion of glycogen synthase to a less active form. PGE_1 (2 or 30 μM) increased cyclic AMP accumulation and activated protein kinase, but caused no detectable changes in *dP/dt* or the activation states of the protein kinase substrates involved in glycogen metabolism. Perfusion of hearts with either 10 nM isoproterenol or 30 μM PGE_1 produced comparable increases in cyclic AMP accumulation and protein kinase activity. Exposure of hearts to a combination of these agents caused additive effects on cyclic AMP content and protein kinase activity. However, values for phosphorylase kinase, glycogen phosphorylase, glycogen synthase, and *dP/dt* did not differ from those observed in the presence of 10 nM isoproterenol alone. The failure of PGE_1 to stimulate phosphorylation of protein kinase substrates was not due to an increase in phosphorylase phosphatase activity. We conclude that an increase in intracellular cyclic AMP and the subsequent activation of protein kinase are insufficient to change either the activities of phosphorylase kinase, glycogen phosphorylase, and glycogen synthase or the inotropic state of heart muscle.**

Kuo and Greengard (1) have advanced the idea that ubiquitous cyclic AMP-dependent protein kinases (ATP:protein phosphotransferase, EC 2.7.1.37) mediate most, if not all, of the effects of cAMP in eukaryotes (1–3). According to this hypothesis the ability of a hormone to elicit a response mediated by cAMP would be determined by the presence of the appropriate adenylate cyclase-linked receptor on the surface of the cell and by the presence within the cell of appropriate substrates for protein kinase. Thus, receptors specify the spectrum of hormonal sensitivity of a cell and available substrates specify the precise response. Much evidence supports the universality of Kuo and Greengard's hypothesis. It is apparent that cAMP is the only ligand necessary for the activation of protein kinase (4, 5). Numerous events, notably glycogenolysis (2) and lipolysis (6), result from phosphorylation of proteins by cAMP-dependent protein kinases subsequent to elevation of intracellular cAMP. Other than phosphodiesterase, the regulatory subunit of protein kinase appears to be the major high-affinity binding protein for cAMP (1). As noted by Rall, however, the protein kinase hypothesis presents us with "the unsatisfying picture of the catalytic subunit of protein kinase swimming about, happily phosphorylating a variety of cellular constituents whether they need it or not" (7).

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Recently, there have been several reports in the literature suggesting that under certain conditions accumulation of cAMP and activation of cAMP-dependent protein kinase are insufficient to affect the activities of several enzymes that are regulated by phosphorylation–dephosphorylation reactions. Keely (8) has demonstrated that prostaglandin E_1 (PGE_1) and epinephrine both produced increases in cAMP and the cAMP-dependent protein kinase activity ratio in the perfused rat heart, but only epinephrine caused a significant increase in glycogen phosphorylase activity. Honeyman and Levy (9) have reported that in adipose tissue epinephrine, isoproterenol, methylisobutylxanthine, dibutyryl-cAMP, and glucagon all increased intracellular cAMP content, activated protein kinase and phosphorylase, and stimulated glycerol production, whereas serotonin increased cAMP accumulation and activated protein kinase but paradoxically increased phosphorylase activity without increasing lipolysis.

We undertook the present study in an attempt to clarify the role of cAMP and cAMP-dependent protein kinase in the regulation of interconvertible enzymes involved in glycogen metabolism. We have investigated the relationship between the effects of isoproterenol and PGE_1 on cAMP accumulation, the activation state of protein kinase, the activation states of two substrates of protein kinase (phosphorylase *b* kinase and glycogen synthase), phosphorylase activity, and the contractile state in perfused rat heart. A comparison of the effects of PGE_1 and isoproterenol on these variables offers some insights into the specificity of hormonal regulation of cardiac contractility and metabolism.

EXPERIMENTAL PROCEDURES

Heart Perfusion. Fed male rats weighing 200–300 g were used. Sodium heparin (1500 units/kg) was injected intraperitoneally 15 min before sacrifice. Hearts were quickly excised from Nembutal-anesthetized animals (100 mg/kg), immersed in ice-cold saline until beating ceased, attached via the aorta to the perfusion canula, and perfused (retrograde) without recirculation at an aortic pressure of 60 mm Hg with Krebs–Henseleit bicarbonate buffer at 32°C equilibrated with 95% O_2/5% CO_2 and containing 10 mM glucose. Left ventricular *dP/dt* (*P*, pressure) was used as a measure of contractile state. This was measured by inserting a 23-gauge needle attached to a P23Gb Statham pressure transducer into the left ventricular cavity. Recordings were made on a Grass model 7D polygraph. Hearts were paced at 240 beats per min, equilibrated for 30 min before drug treatment, and then perfused for 2 min with buffer containing drug. After the treatment period, hearts were quickly frozen with Wollenberger clamps that had been cooled in liquid nitrogen. The atria and great vessels were trimmed

Abbreviations: cAMP, cyclic AMP; PGE_1, prostaglandin E_1; Glc-6-*P*, glucose 6-phosphate; *P*, pressure.

away, and the remaining tissue was pulverized to a fine powder with a percussion mortar that had also been cooled in liquid nitrogen. The powder was stored at −70°C until assayed (usually within 7 days). There was no loss in enzyme activity or degree of activation of protein kinase, glycogen synthase, phosphorylase kinase, or glycogen phosphorylase even after 2 months of storage.

cAMP Assay. Approximately 40 mg of powdered tissue was homogenized in 0.5 ml of ice-cold 10% trichloroacetic acid containing 0.25 pmol of [^{3}H]cAMP (8000 cpm) to determine the percentage of cAMP recovered after purification. The deproteinized extract was purified over Dowex 50 AG WX-4 (200–400 mesh). cAMP was quantified by the protein binding assay of Gilman (10). Protein pellets were dissolved in NaOH; protein was quantified by the method of Bradford (11). Values are expressed as pmol of cAMP per mg of protein.

Protein Kinase Assay. Approximately 30 mg of powdered tissue was suspended at 4°C in 10 vol of 10 mM potassium phosphate buffer (pH 6.8) containing 10 mM EDTA and 0.5 mM 1-methyl-3-isobutylxanthine, and homogenized with a motor-driven ground glass pestle in a ground glass tube. The homogenate was immediately centrifuged at 20,000 × *g* for 15 min at 4°C, and the supernatant was used for the assay. The protein kinase assay was based on the phosphorylation of histone and was carried out as described by Keely *et al.* (12). The kinase activity is expressed as the protein kinase activity ratio (the ratio of activity in the absence of cAMP to that in the presence of 2 μM cAMP). Changes in the activity ratio occurred without changes in total activity.

Phosphorylase Kinase Assay. Approximately 30 mg of powdered tissue was suspended at 4°C in 10 vol of 30 mM Tris·HCl, pH 7.5/30 mM KCl/5 mM EDTA/100 mM NaF/1 mM phenylmethylsulfonyl fluoride/0.02% bovine serum albumin and homogenized with a motor-driven ground glass pestle in a ground glass tube. The homogenate was immediately centrifuged at 20,000 × *g* for 20 min at 4°C, the resulting supernatant being the source of the enzyme. Phosphorylase kinase activity was determined by measuring the incorporation of ^{32}P from [γ-^{32}P]ATP into purified rabbit skeletal muscle phosphorylase *b*. The standard reaction mixture contained 20 μl of 0.125 M Tris/0.125 M β-glycerophosphate, 20 μl of phosphorylase *b* (30 mg/ml, 95 μM final concentration), 3.0 mM ATP (60 cpm/pmol), 10 mM magnesium acetate and 10 μl of a 1:10 (pH 6.8) or 1:20 (pH 8.2) dilution of heart cytosol in a total volume of 63 μl. The reaction tubes were warmed to 30°C, and the assay was initiated by the addition of Mg^{2+}–ATP to the mixture. The reaction was terminated by pipetting a 55-μl aliquot of the reaction mixture onto a Whatman 3 MM filter paper disc which was dropped into ice-cold 10% trichloroacetic acid containing 2% sodium pyrophosphate. Discs were washed (10 ml per disc) as follows: 15 min in 10% trichloroacetic acid/2% sodium pyrophosphate; 30 min in 5% trichloroacetic acid/1% sodium pyrophosphate; 2 × 45 min in 5% trichloroacetic acid/1% sodium pyrophosphate; and 5 min in 95% ethanol. Discs were dried with a heat lamp and assayed for radioactivity by liquid scintillation spectrometry in toluene-containing Omnifluor (4 g/liter). Phosphorylase kinase data are presented as the ratio of activity at pH 6.8 to that at pH 8.2. This has been shown to be a valid index of the amount of enzyme present in the active form in rat heart (13).

Glycogen Phosphorylase Assay. Glycogen phosphorylase was assayed in both the presence and absence of AMP by a described fluorometric method (14). The results are expressed as the ratio of phosphorylase activity without AMP to phosphorylase activity with AMP. An increase in the ratio indicates an increase in the amount of phosphorylase in the *a* form.

Phosphorylase Phosphatase Assay. For assay of phosphatase activity, heart powder was homogenized (1:5) in 50 mM Tris·HCl, pH 7.6/15 mM 2-mercaptoethanol/1 mM phenylmethylsulfonyl fluoride/2 mM EDTA/0.2 mM EGTA with charcoal (10 mg/ml) and centrifuged for 20 min at 20,000 × *g*. The supernatant was combined with glycogen phosphorylase *a* (1 mg/ml) and 7 mM $MnCl_2$ (final concentrations) and incubated at 30 °C. Aliquots were removed at appropriate times and assayed for phosphorylase activity as described above. β-Glycerol-*P* (50 mM) and 20 mM NaF were used as phosphatase inhibitors in several experiments.

Glycogen Synthase Assay. Glycogen synthase was assayed by the filter paper method of Thomas *et al.* (15). Frozen powered tissue was homogenized in 50 vol of buffer (5 mM EDTA/50 mM KF/0.25 M sucrose/0.01% bovine serum albumin/1 mM phenylmethylsulfonyl fluoride/50 mM Tris·HCl, pH 7.8); the 100,000 × *g* min supernatant was diluted 1:3 in buffer. Reactions were intiated by adding 50 μl of this extract to reaction mixtures containing 0.5 mM [^{14}C]UDP-Glc (1 μCi/μmol; 1 Ci = 3.7 × 10^{10} becquerels), 1 mM EDTA, 0.8% oyster glycogen, 25 mM Tris·HCl at pH 7.8, and appropriate concentrations (0–10.3 mM) of glucose 6-phosphate (Glc-6-*P*), in a total volume of 125 μl. The half maximally effective concentration ($A_{0.5}$) of the modulator Glc-6-*P* was determined from Hill plots as described (16). For determination of the activity ratio (−Glc-6-*P* to +Glc-6-*P*), UDP-Glc was at 5 mM and a 1:40 dilution of tissue was used, with 0 and 10.3 mM Glc-6-*P*.

Materials. Phosphorylase *a* was prepared from rabbit skeletal muscle by the method of Fischer and Krebs (17). John Pike at Upjohn donated PGE_1. All other reagents were obtained from standard commercial sources.

RESULTS

Our previous results demonstrated that PGE_1 and isoproterenol act differently on the glycogenolytic cascade of rat heart (18). To investigate the basis of this difference, we employed the β-adrenergic agonist isoproterenol to avoid any complication with α-adrenergic effects produced by epinephrine. In our initial experiments, we compared the effects of isoproterenol (80 nM) and PGE_1 (2 μM) on cAMP content and protein kinase activity (Table 1). The drugs gave qualitatively similar effects. Both agents increased intracellular cAMP and caused the activation of protein kinase. To determine at what stage of the biochemical events associated with glycogenolysis the responses to isoproterenol and PGE_1 differed, we examined the activities of two substrates for protein kinase, phosphorylase kinase, and glycogen synthase. We also assessed the activity of glycogen phosphorylase, the substrate for phosphorylase kinase, because modest changes in the activation state of phosphorylase kinase are enzymically magnified in the activation state of phosphorylase (19, 20). Since increased contractility has been associated with the phosphorylation of troponin I by cAMP-dependent protein kinase (21), we monitored left ventricular

Table 1. Effects of isoproterenol and PGE_1 on cAMP content and protein kinase activity

Treatment	cAMP, pmol/mg protein	Protein kinase, −cAMP/+cAMP
Control	5.0 ± 0.2	0.17 ± 0.01
Isoproterenol (80 nM)	9.7 ± 0.7**	0.39 ± 0.01**
PGE_1 (2 μM)	6.5 ± 0.4*	0.24 ± 0.01**

Values are mean ± SEM of determinations from 8–11 hearts. Asterisks indicate values significantly different from control by unpaired *t* test analysis (*, $P < 0.01$; **, $P < 0.001$).

Table 2. Effects of isoproterenol and PGE_1 on the enzymes regulating glycogen metabolism and on *dP/dt*

Treatment	Phos kinase, pH 6.8:8.2	Phosphorylase, −AMP/+AMP	Glycogen synthase, $A_{0.5}$, mM	*dP/dt*
Control	0.14 ± 0.01	0.05 ± 0.02	0.42 ± 0.04	1.0
Isoproterenol (80 nM)	0.19 ± 0.01*	0.58 ± 0.03**	0.82 ± 0.09*	1.7**
PGE_1 (2 μM)	0.14 ± 0.01	0.06 ± 0.01	0.39 ± 0.04	1.0

Values are mean ± SEM of determinations from 8–11 hearts. Asterisks indicate values significantly different from control by unpaired *t* test analysis (*, $P < 0.005$; **, $P < 0.001$). *dP/dt* is expressed as fraction of control (1680 ± 60 mm Hg/sec).

dP/dt as a measure of another potential "substrate" for protein kinase. Table 2 summarizes the results of these studies.

Isoproterenol caused the well-known and anticipated alterations: activation of phosphorylase kinase, reflected in the conversion of phosphorylase *b* to *a*; conversion of glycogen synthase to a less active form; and enhanced rate of ventricular pressure generation. It is noteworthy that the decrease in the apparent affinity of glycogen synthase for its allosteric modifier (Glc-6-*P*) occurred without changes in the −Glc-6-*P* to +Glc-6-*P* activity ratio (average −Glc-6-*P* to +Glc-6-*P* ratio, 0.08 ± 0.02; no significant changes with drug treatment). Indeed, a decrease from the low control ratio would be extremely difficult to detect by the Glc-6-*P* ratio method. The increase in $A_{0.5}$ for Glc-6-*P* in the face of an unchanged activity ratio reinforces our earlier conclusion that the apparent affinity of glycogen synthase for its allosteric modulator is a more sensitive index of the enzyme's activation state and extent of phosphorylation than is the −Glc-6-*P* to +Glc-6-*P* activity ratio (16).

In contrast to isoproterenol, PGE_1 caused no detectable changes in the activities of phosphorylase kinase, phosphorylase, and glycogen synthase, and no alteration in inotropic state. Thus, in spite of the enhancement of intracellular cAMP content and the activation of protein kinase by PGE_1 (Table 1), there was a failure of the activation of protein kinase to be expressed. These data show that PGE_1 does not cause phosphoryase *b* to *a* conversion and demonstrate that the explanation of this observation seems to be the failure of protein kinase to act on its substrate, phosphorylase kinase. The lack of changes in glycogen synthase and *dP/dt* support the hypothesis that PGE_1 causes an ineffective activation of cardiac protein kinase with respect to its ability to phosphorylate several of its substrates.

The comparison of the actions of isoproterenol and PGE_1 offered by Tables 1 and 2 has several notable limitations. The extents of cAMP accumulation and protein kinase activation in response to the two agonists are qualitatively but not quantitatively comparable. Conceivably, the modest activation of protein kinase by PGE_1 was insufficient to cause subsequent protein phosphorylation of substrates that we measured. In order to compare the two hormones under conditions in which they produced similar increases in protein kinase activity, we perfused rat hearts with a lower concentration of isoproterenol (10 nM) and a higher concentration of PGE_1 (30 μM). We also examined the possibility that PGE_1 might specifically inhibit the catalytic function of protein kinase by testing the two agents in combination.

The results of these experiments are shown in Table 3. Both 10 nM isoproterenol and 30 μM PGE_1 caused similar increases in cAMP accumulation and activation of protein kinase. However, only isoproterenol increased *dP/dt*, activated phosphorylase kinase and phosphorylase, and inactivated glycogen synthase. PGE_1 did not block the isoproterenol-induced increase or decrease in these enzyme activities or *dP/dt*. We have expressed the results of Table 3 as percentages of the maximal (10 nM isoproterenol plus 30 μM PGE_1) values and plotted them in Fig. 1 in a manner similar to a metabolic crossover plot. This plot visually emphasizes the parallel and divergent aspects of several experimental conditions and does

Table 3. Effects of isoproterenol and PGE_1 alone and in combination on cardiac function and metabolism

	Control	PGE_1	Isoproterenol	Isoproterenol and PGE_1
cAMP (pmol/mg protein)	5.0 ± 0.2	7.3 ± 0.3**	8.6 ± 0.5**	11.2 ± 0.7**
Protein kinase (−cAMP/+cAMP)	0.17 ± 0.01	0.24 ± 0.02**	0.26 ± 0.01**	0.33 ± 0.01**
Phosphorylase kinase (pH 6.8:8.2)	0.145 ± 0.01	0.15 ± 0.01	0.17 ± 0.01*	0.18 ± 0.01*
Phosphorylase (−AMP/+AMP)	0.05 ± 0.02	0.08 ± 0.03	0.36 ± 0.02**	0.34 ± 0.03**
Glycogen synthase ($A_{0.5}$, mM)	0.43 ± 0.04	0.43 ± 0.05	0.85 ± 0.08**	0.77 ± 0.08**
dP/dt	1.0	0.94	1.77**	1.83**

Values are mean ± SEM of determinations from 4–11 hearts. Asterisks indicate values differing significantly from control values by unpaired *t* test analysis (*, $P < 0.04$; **, $P < 0.001$). *dP/dt* is expressed as fraction of control (1680 ± 60 mm Hg/sec). Values for cAMP and protein kinase with 30 μM PGE_1 are not statistically different from those with 10 nM isoproterenol. Values for phosphorylase kinase, phosphorylase, glycogen synthase, and *dP/dt* in the presence of 10 nM isoproterenol do not differ significantly from those with both agents, whereas values for cAMP and protein kinase in the presence of both agents are significantly greater than with either agent alone ($P < 0.02$).

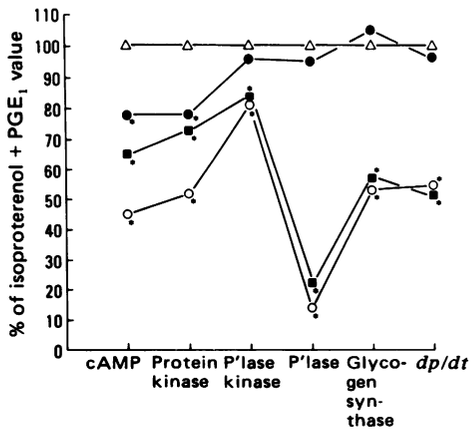

FIG. 1. Divergent effects of isoproterenol and PGE_1 on several metabolic variables. *, $P < 0.01$ as compared to isoproterenol + PGE_1 values. Refer to text and to Table 3 for details. ○, Control; ■, PGE_1; ●, isoproterenol; △, isoproterenol and PGE_1. P'lase, phosphorylase.

not imply a cause–effect relationship. Again, it can be seen that isoproterenol (10 nM) and PGE_1 (30 μM) produced comparable elevations of cAMP and protein kinase activity. When the agents were used in combination, their effects on cAMP content and protein kinase activation were additive. Beyond protein kinase in the biochemical sequence (moving to the right on the abscissa in Fig. 1), the effects of PGE_1 and isoproterenol diverged. The values for phosphorylase kinase, phosphorylase, glycogen synthase, and dP/dt in response to isoproterenol alone did not differ significantly from those due to the combination of isoproterenol and PGE_1. For the same variables, the effect of PGE_1 precisely paralleled the control values. Thus, with comparable effects on cAMP accumulation and protein kinase activation, PGE_1 and isoproterenol still had divergent effects on reactions subsequent to the activation of protein kinase. Even a high concentration of PGE_1 (30 μM) did not cause the expression of protein kinase activity, nor did 30 μM PGE_1 inhibit the effects of a submaximally effective concentration of isoproterenol.

It also seemed possible that isoproterenol and PGE_1 could be acting on separate cell populations, each with responsiveness

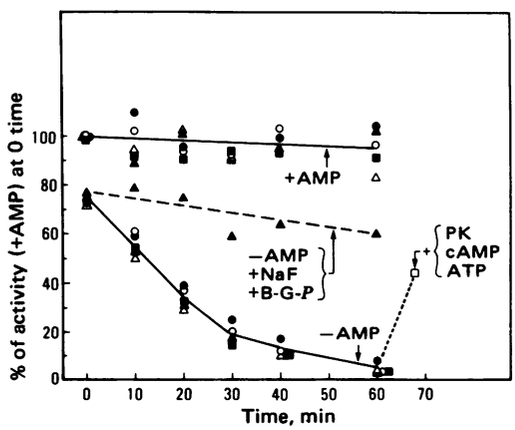

FIG. 2. Phosphorylase *a* phosphatase activity. Frozen heart powders from the indicated treatment groups were assayed for phosphatase activity. Total activity (+AMP, 2 mM) was constant. Addition of NaF (20 mM) and β-glycerol-*P* (B-G-*P*, 50 mM) prevented decline of phosphorylase *a* activity (−AMP). Addition of cyclic AMP (20 μM), methylisobutylxanthine (50 μM), ATP (1.4 mM) and an excess of protein in kinase (PK) (300 units) at 60 min restored phosphorylase *a* activity. ●, Isoproterenol; △ ▲ ○, PGE_1; ■, control.

to one of the hormones. Treatment of perfused hearts with the combination of maximal concentrations of isoproterenol (100 nM) and PGE_1 (30 μM) did not, however, produce more cAMP accumulation or activation of protein kinase than 100 nM isoproterenol alone (data not shown). Some additivity of maximal effects would be expected were several cell populations involved, whereas our result of nonadditivity agrees with the usual observation for a cell with multiple receptors linked to adenylate cyclase (22).

We have considered that the failure of PGE_1 to cause phosphorylative events subsequent to the activation of protein kinase could be due to a generalized increase in phosphoprotein phosphatase by PGE_1. Thus, activation of enzymes by phosphorylation would occur but be counterpoised by dephosphorylation. To test this possibility, we assayed phosphatase activities in soluble fractions from frozen powders of control and treated hearts, using phosphorylase *a* as a substrate. Fig. 2 summarizes these data and shows that phosphorylase *a* activity was decreased at the same rate by extracts from all heart samples, regardless of drug treatment. The phosphatase inhibitors NaF and β-glycerol-*P* prevented the decrease in phosphorylase *a* activity. Total phosphorylase activity (+AMP) was constant throughout the assay. The addition of cAMP and cAMP-dependent protein kinase at 60 min restored phosphorylase *a* activity as determined in the absence of its allosteric activator, AMP. These results indicate that the decreases in phosphorylase *a* activity depicted in Fig. 2 were due to the action of phosphatases.

DISCUSSION

We have compared the effects of isoproterenol and PGE_1 on glycogen metabolizing enzymes and dP/dt in Langendorff perfused rat hearts. Perfusion of hearts with isoproterenol (10 or 80 nM) caused enhancement of dP/dt, increased intracellular cAMP, and caused increases in the activation states of protein kinase, phosphorylase kinase, and phosphorylase, and the conversion of glycogen synthase to a less active form. By contrast, PGE_1 (2 or 30 μM) increased cAMP accumulation and the protein kinase activity ratio, but caused no detectable changes in the activities of two protein kinase substrates (phosphorylase kinase and glycogen synthase) and neither activation of glycogen phosphorylase nor alteration of left ventricular dP/dt. When cAMP accumulation and protein kinase activation were stimulated by PGE_1, there was a failure of the protein kinase activation to be expressed. We have tested several hypotheses which could explain the anomalous effects of PGE_1. We considered that the poorer efficacy of PGE_1 that led to smaller effects than those of isoproterenol might contribute to our observations. However, when the concentrations of PGE_1 and isoproterenol were adjusted to produce comparable effects on cAMP content and protein kinase activation, the anomaly persisted. Possibly PGE_1 was supplying an inhibitory influence or factor such as the increased availability of the heat-stable inhibitor of protein kinase. Were this the case, PGE_1 should cause a diminution of the effects of isoproterenol. When we perfused rat hearts with a combination of PGE_1 and isoproterenol, we observed no inhibition of any of the effects of the β-agonist. A submaximal concentration of isoproterenol (10 nM) produced the full complement of glycogenolytic activations and an increased rate of force development even in the presence of a high concentration of PGE_1 (30 μM) (Fig. 1). We have also tested the possibility that PGE_1 activated a phosphatase that just balanced the catalytic activity of protein kinase so that no net phosphorylation occurred. To the extent that phosphorylase *a* phosphatase is an appropriate measure, our results (Fig. 2) indicate no stable activation of phosphoprotein phosphatase activity by the prostaglandin.

We cannot unequivocally rule out the existence of several responsive cell types in such an experiment. Were there two ventricular cell populations with different hormonal responses, we would still be forced to postulate a cell type without the substrates for protein kinase normally involved in glycogen metabolism. The data from experiments involving perfusion of hearts with combinations of PGE_1 and isoproterenol also address the possibility of multiple cell types. Submaximal, but not maximal, concentrations of PGE_1 and isoproterenol caused additive accumulation of cAMP and protein kinase via a single, limiting, hormone-sensitive population of adenylate cyclase. The reason for the failure of PGE_1 to potentiate the effects of submaximal isoproterenol on protein phosphorylation is unclear, because the combination of these agents is additive with respect to cAMP accumulation and protein kinase activation (Table 3 and Fig. 1). If both isoproterenol and PGE_1 were activating identical fractions of protein kinase, one might expect a combination of these agents to produce additive effects on the phosphorylation of protein kinase substrates. Recently Corbin *et al.* (23) have reported that isozymes of cAMP-dependent protein kinase are compartmentalized in heart tissue. It is possible that we are observing the selective activation of specific pools of protein kinase by isoproterenol and PGE_1.

Several investigators have correlated cAMP accumulation and activation of protein kinase with a positive inotropic response (24–26). We conclude from our results that the selective activation of protein kinase caused by PGE_1 is insufficient to stimulate inotropy or that activation of protein kinase is, in general, inadequate for or unrelated to enhanced contractility. Perhaps inotropic and glycogenolytic agents that stimulate cAMP accumulation also provide a factor necessary for inotropy that isoproterenol but not PGE_1 supplies. In the present studies, contractility was measured by the assessment of left ventricular pressure development. This method is considerably more sensitive than those of aortic or left ventricular pressure that were used in studies showing highly variable inotropic effects of PGE_1 (27). Our data on the glycogenolytic events suggest that Ca^{2+} is not the missing factor; the apparent blockade is in the function of protein kinase, an enzyme not known to require Ca^{2+}. We cannot rule out the possibility that isoproterenol selectively influences some other essential event.

We are left with an enigma: two agonists, isoproterenol and PGE_1, that share several primary characteristics lead to very different results. Both agents interact with membrane-bound receptors to stimulate adenylate cyclase (28) and cause consequent activation of protein kinase. Both drugs probably act on membranes to influence events other than cAMP production (29, 30). However, only isoproterenol causes the events that result from protein phosphorylation; protein kinase activation resulting from treatment with PGE_1 does not affect several well-known substrates of the kinase in heart. Thus, our data provide evidence that protein phosphorylation following increased intracellular cAMP can be hormonally specific. These results do not support a generalized activation of protein kinase as the sole mediator of cAMP's actions, as proposed by Kuo and Greengard (1). A more complex model is required to provide for the apparent hormonal specificity of expression of protein kinase activity that we and others have observed (8, 9) in several experimental systems with PGE_1 and serotonin.

We have not yet explained this paradox but we do have several hypotheses amenable to testing: (*i*) isoproterenol may supply an unidentified "factor" necessary for the expression of protein kinase activity, though, as discussed above, we do not feel that Ca^{2+} is that factor; (*ii*) isoproterenol, but not PGE_1, may translocate catalytic subunits of protein kinase, resulting in the phosphorylation of the glycogen regulatory enzymes; (*iii*) isoproterenol may lead to the selective activation of a protein kinase isozyme associated with the glycogen particle that contains most of the cellular glycogen synthase and phosphorylase kinase (31), the major substrates of protein kinase that we have measured; (*iv*) the two hormones may activate discrete pools of protein kinase. The finding of Corbin *et al.* (23) that isozymes of protein kinase are distributed within distinct cellular compartments is important to these hypotheses. It is reasonable to suppose that these isozymes are under different types of regulation, have distinct functions, and are associated with different substrates.

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