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Prostaglandin E_1 Activation of Heart cAMP-dependent Protein Kinase: Apparent Dissociation of Protein Kinase Activation from Increases in Phosphorylase Activity and Contractile Force

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SUMMARY

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The effects of prostaglandin E_1 (PGE_1) on cAMP, cAMP-dependent protein kinase, glycogen phosphorylase, and contractile force have been investigated in the isolated perfused rat heart, and compared to the effects of epinephrine. In this heart preparation both PGE_1 and epinephrine produced rapid, concentration-dependent increases in cAMP and the cAMP-dependent protein kinase activity ratio. When dosages were adjusted to give equal increases in the protein kinase activation ratio from a basal value of 0.15 to as high as 0.40, only epinephrine produced a significant increase in contractile force or phosphorylase activity. Neither the α-adrenergic agonist phenylephrine nor ionophore A-23187 altered the inability of PGE_1 to augment phosphorylase activity or force. The phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine potentiated the effect of PGE_1 on cAMP and protein kinase activity. When used at concentrations of 10 μM or less, PGE_1 failed to increase phosphorylase kinase activity even though it did produce a 2-fold increase in the cAMP-dependent protein kinase activity ratio. If very high protein kinase activity ratios were generated by using very high PGE_1 levels (100 μM) or PGE_1 in combination with isobutylmethylxanthine, increases in phosphorylase kinase activity were observed. This activation was, however, less than that observed when epinephrine was used to produce a similar protein kinase activation state and was accompanied by a slight increase in phosphorylase activity. When used together, PGE_1 and epinephrine produced partially additive effects on cAMP and protein kinase activity and approximately the same increase in phosphorylase activity as did epinephrine when used alone. If very high cAMP levels or protein kinase activity ratios were produced by infusion of epinephrine plus 3-isobutyl-1-methylxanthine, PGE_1 produced no further increase in either parameter.

INTRODUCTION

It is now generally accepted that cyclic adenosine 3':5'-monophosphate (cAMP)[1] mediates the effects of many cardioactive agents on heart metabolism and function (1-4). It has been postulated that many of the effects of cAMP in mammalian tissues are due to changes in the activity of cAMP-dependent protein kinase(s) (5). Work from this laboratory has demonstrated that in the isolated perfused heart many hormones

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[1] The abbreviations used are: cAMP, cyclic adenosine 3':5'-monophosphate; PGE_1, prostaglandin E_1; and TCA, trichloroacetic acid.

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and drugs that increase cAMP also activate the heart protein kinases (6–8). Recent evidence has suggested an important role for these kinases in the regulation of cardiac glycogen and lipid metabolism and contractile force (6–13).

In all reports to date, any agent that has produced an increase in the heart cAMP-dependent protein kinase activity ratio from the basal value of 0.15 to a value of 0.22 or greater has also significantly increased glycogen phosphorylase activity and contractile force (6, 9, 12, 14). In this paper, a possible dissociation between increases in the heart cAMP-dependent protein kinase activity ratio and changes in either phosphorylase activity or contractile force is reported.

EXPERIMENTAL PROCEDURES

Heart perfusion. Fed male rats weighing 150–160 g were used. Sodium heparin (1500 U/kg) was injected intraperitoneally 15 min before sacrifice. The animals were anesthetized with sodium pentobarbital (100 mg/kg). Hearts were quickly excised, immersed in cold saline (4°) until beating ceased, attached via the aorta to a perfusion cannula, and perfused at 37° with recirculating Krebs-Henseleit bicarbonate buffer (8 ml/min) containing 1.25 mM Ca^{++} and 10 mM glucose. The buffer was equilibrated with O_2/CO_2 (95/5%). Force of contraction was estimated by measuring peak tension development by a Statham strain-gauge transducer attached to the apex of the heart by a stainless steel suture. Diastolic tension was set at 2.5 g. Tension development was recorded on a Sanborn 964 recorder. In all cases, hearts were electrically paced at 280 beats/min by a Grass S9 stimulator. At the end of the perfusion period, hearts were quickly frozen between Wollenberger clamps which had been cooled in liquid nitrogen. The atria and great vessels were trimmed away and the remaining tissue pulverized to a fine powder with a percussion mortar which had also been cooled in liquid nitrogen. The powders were stored at −70° until assayed. All assays occurred within 48 hr of the perfusion time.

Protein kinase assay. Approximately 40 mg of powdered tissue were suspended at 4° in 50 times the weight of 10 mM potassium phosphate–10 mM EDTA–0.5 mM 3-isobutyl-1-methylxanthine buffer (pH 6.8) and homogenized with three up and down strokes of a motor-driven Teflon pestle in a glass tube homogenizer. Tissue homogenization at this weight:volume ratio greatly reduced translocation of the free catalytic subunit of the protein kinase to particulate material, thus minimizing the possibility of an artifactual lowering of supernatant kinase activity (15). The homogenate was immediately centrifuged at 27,000 × *g* for 20 min. The protein kinase assay was started by adding 10 μl of the supernatant fraction to 50 μl of a solution containing 17 mM potassium phosphate (pH 6.8), 0.33 mM[γ-^{32}P]-ATP (~500 cpm/pmol), 6 mM magnesium acetate, and 0.5 mg histone (Sigma type II-A) in the absence or presence of 2 μM cAMP. The incubation was carried out at 30° for 5 min and terminated by pipetting a 50 μl aliquot of the reaction mixture onto a filter paper disc (1 × 2 cm), which was immediately dropped into 10% TCA. The filter papers were washed 4 times for 15 min each in TCA and one time each for 5 min in 95% ethanol and diethyl ether. The papers were air-dried and counted in 10 ml of toluene-Cellosolve scintillation fluid. Cyclic AMP-dependent protein kinase activity is expressed as the activity ratio, i.e., the ratio of activity in the absence of added cAMP to that in the presence of enough exogenous cAMP (2 μM) to maximally activate the enzyme. No treatment procedures used in this study produced a change in total cAMP-dependent protein kinase activity (+ cAMP).

Cyclic AMP assay. Cyclic AMP was estimated by the protein binding assay of Gilman (16). Approximately 100 mg of powdered heart tissue were homogenized in 1 ml of 5% TCA containing 0.1 pmol of [^{3}H]-cAMP (50,000 cpm/pmol) to determine the percentage of cAMP recovered after purification. The deproteinized homogenate was centrifuged at 27,000 × *g* for 20 min. The supernatant fraction was then pipetted onto a Dowex 50 column (0.9 × 10 cm; 100 to 200 mesh) equilibrated with 0.1 N HCl. The column was eluted with 30 ml of 0.1 N

HCl with collection of the last 20 ml. This solution was lyophilized and resuspended in 1 ml of 50 mM sodium acetate (pH 4.0). The concentrations of cAMP in these solutions were determined against known standards using Millipore filtration.

Phosphorylase activity. Phosphorylase activity was determined by measuring the incorporation of [^{14}C]glucose-6-phosphate into glycogen (17). Approximately 30 mg of heart tissue were homogenized at −30° in 0.5 ml of a 60% glycerol buffer containing 10 mM glycerophosphate, 10 mM EDTA, 20 mM NaF, and 50 mM β-mercaptoethanol. The homogenate was then diluted 3-fold with a buffer identical to that mentioned above except for the absence of glycerol. The diluted homogenate was centrifuged at 27,000 × *g* for 20 min. The assay reaction was started by adding 35 μl of the supernatant fraction to 35 μl of a solution containing 2% glycogen and 32 mM [U-^{14}C]glucose-6-phosphate in the presence and absence of 3 mM AMP. The reaction mixture was incubated at 30° for 10 min and then terminated by pipetting a 50 μl aliquot onto a 1 × 2 cm filter paper disc (Whatman 3MM) which was immediately dropped into 66% ethanol. The papers were washed three times in ethanol for 30 min each, dried, and counted in toluene-Cellosolve scintillation fluid. Phosphorylase activity is expressed as the ratio of the activity in the absence to that in the presence of AMP. There was no change in total phosphorylase activity (a + b) in response to any of the drugs used in this investigation.

Phosphorylase b kinase. Phosphorylase b kinase activity was determined as described by Krebs *et al.* (18) as modified by Drummond and Duncan (19). All hearts were assayed in duplicate. Results are expressed as the ratio of phosphorylase a produced at pH 6.8 to that produced at pH 8.2.

Materials. Type II-A histone, cAMP and epinephrine (free base) were from Sigma Chemical Co. [γ-^{32}P]ATP was prepared by the method of Glynn and Chappell (20). PGE_1 was a gift from Dr. John Pike of Upjohn and 3-isobutyl-1-methylxanthine was a gift from Searle. All drug solutions were prepared fresh daily.

RESULTS

Concentration dependence. Figure 1 shows the effect of increasing PGE_1 and epinephrine concentrations on cAMP levels, protein kinase and phosphorylase activities, and contractile force in the perfused rat heart. Force measurements and freezing for subsequent assays were accomplished after a 2 min constant infusion of either drug. Both agents produced concentration-dependent increases in cAMP and cAMP-dependent protein kinase activity (panels A and B). Epinephrine was 10- to 50-fold more potent than PGE_1 in increasing either of these parameters. While epinephrine was capable of producing almost complete activation of the rat heart protein kinase, PGE_1, at the concentrations used, never activated more than 50% of the total amount of the enzyme(s) present.

As we have reported earlier (6, 9), epinephrine infusion produced increases in phosphorylase activity and contractile force, which correlated well with changes in the protein kinase activity ratio. Unlike epinephrine, PGE_1, at the concentrations of 10 μM or less, had little or no effect on either phosphorylase (panel C) or force (panel D), even though it produced a large increase in the protein kinase activity ratio. With very high PGE_1 concentrations (100 μM), small increases in both phosphorylase activity and force were produced. These increases were always less than that observed when epinephrine was used to generate similar cAMP-dependent activity ratios. When added to the protein kinase assay, the cAMP-dependent protein kinase inhibitor described by Walsh *et al.* (21) completely blocked all measured increases in kinase activity in hearts treated with either agent. Addition of charcoal to the homogenization buffer did not alter the activation state of phosphorylase. This would exclude the possibility that the activation of phosphorylase might be due to carry-over of AMP (generated during the inotropic response) into the phosphorylase assay.

Time course of activation. As is shown in Figure 2, hearts perfused with a constant, submaximal concentration of PGE_1 (5 μM) or epinephrine (0.1 μM) showed a rapid increase in cAMP and protein kinase activ-

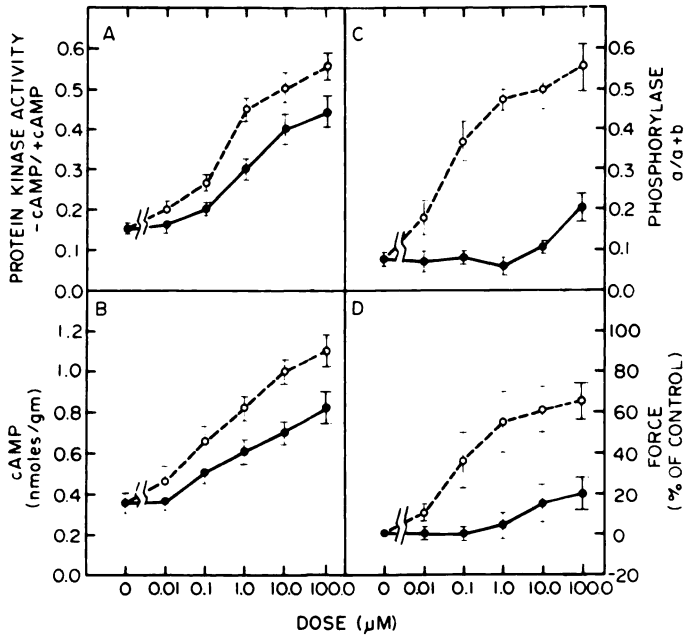

FIG. 1. *Effect of PGE_1 and epinephrine concentration on the protein kinase activity ratio (A), cAMP (B), phosphorylase (C), and contractile force (D) in the perfused rat heart*

Hearts were perfused for 15 min with buffer alone and for an additional 2 min with the indicated concentration of PGE_1 (●——●) and epinephrine (○——○). Handling of the tissue for assay is described under EXPERIMENTAL PROCEDURES. Each point represents the mean ± SEM for 4 to 10 hearts.

ity, reaching a maximum level within 1-2 min (panels A and C). Associated with the epinephrine-induced increase in cAMP and protein kinase activity was a 5- to 6-fold increase in phosphorylase. Although the time course and degree of protein kinase activation produced by 5 μM PGE_1 were similar to those obtained for (0.1 μM) epinephrine, PGE_1 treatment with 5 μM PGE_1 caused no significant increase in phosphorylase activity at any time tested.

Effect of PGE_1 on epinephrine activation. To determine if PGE_1 possessed some activity which inhibited the action of protein kinase on the phosphorylase cascade, hearts were treated for 2 min with either epinephrine alone or in combination wtih PGE_1 (5 μM). As is shown in Figure 3, submaximal concentrations of PGE_1 and epinephrine produced additive effects on protein kinase activation and cAMP. PGE_1 did not alter the ability of epinephrine to activate phosphorylase.

If very high cAMP levels and protein kinase activity ratios were produced by treating hearts with 100 μM epinephrine or 0.1 μM epinephrine plus the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine, PGE_1 did not produce any further increase in either parameter (Table 1). If a maximal concentration of PGE_1 was used (100 μM), epinephrine produced a further elevation of cAMP and protein kinase activity. Epinephrine at high concentrations (100 μM) is capable of nearly completely activating the protein kinase, and thus it is not surprising that PGE_1 produced no further effect on this enzyme. Maximal doses of epinephrine and PGE_1, when added together, have the same increase in cAMP as did epinephrine when used alone (Table 1).

Effects of 3-isobutyl-1-methylxanthine on PGE_1 activation. Figure 4 shows the effect of the cyclic nucleotide phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (1 μM) on the PGE_1 dose response. At the concentration and time used, the methylxanthine had only slight, if any effect by itself (see Table 4), but did potentiate the effects of PGE_1 (compare Figs. 1 and 4).

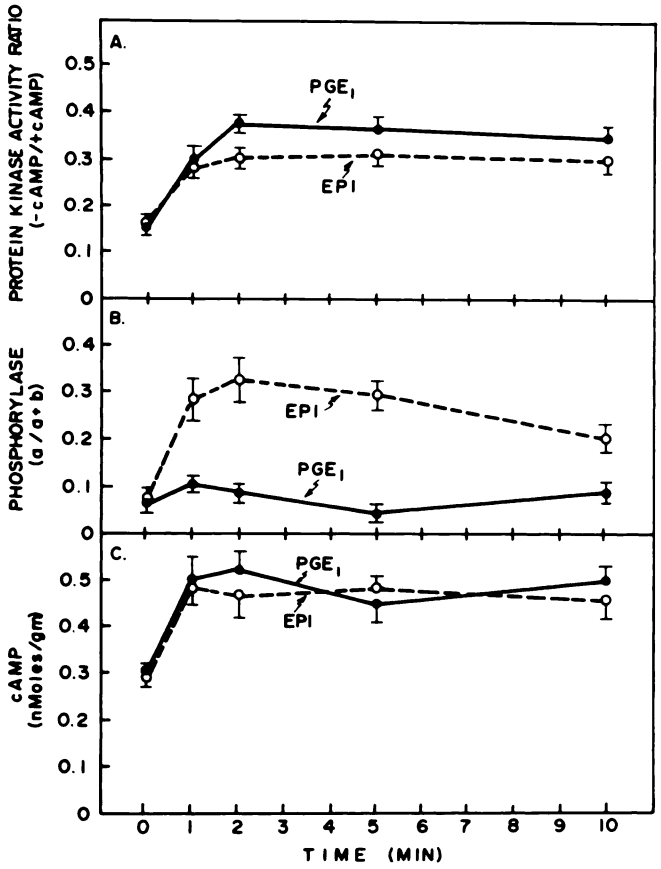

FIG. 2. *Time course of the effect of PGE_1 and epinephrine on protein kinase activity ratio (A), phosphorylase (B), and cAMP (C)*

Hearts were perfused for 15 min with buffer alone and then for the additional time indicated with 5 μM PGE_1 (●——●) or 0.1 μM epinephrine (○——○). Hearts were processed and assayed as described under EXPERIMENTAL PROCEDURES. Each point represents the mean ± SEM for 10 hearts.

Although a high degree of protein kinase activation was obtained using PGE_1 in combination with isobutylmethylxanthine, little or no increase in phosphorylase activity or force was observed. Epinephrine (0.1 μM), while generating lower cAMP levels and protein kinase activities than did the PGE_1-isobutylmethylxanthine combination, produced a large increase in phosphorylase and contractile force (Fig. 4).

Effect of α-adrenergic stimulation and ionophore A23187 on PGE_1 activation. In an effort to determine if PGE_1 in combination with agents which have the potential of increasing intracellular calcium concentrations might act to produce increases in phosphorylase activity and force similar to those observed with epinephrine, hearts were perfused with PGE_1 plus either phenylephrine or ionophore A-23187. As shown in Table 2, PGE_1 in combination with the α-adrenergic agonist phenylephrine produced no significant increase in phosphorylase activity even though protein kinase was highly activated. As has been observed with other agents which activated cardiac adenylate cyclase (8, 22), PGE_1-induced increases in cAMP and protein kinase appeared to be slightly antagonized by concurrent α-adrenergic stimulation.

Table 3 shows the effects of PGE_1 together with the divalent cation ionophore A-23187 on cAMP, protein kinase, phosphorylase, and force. The ionophore attenuated the ability of PGE_1 to increase cAMP and protein kinase activity (compare addi-

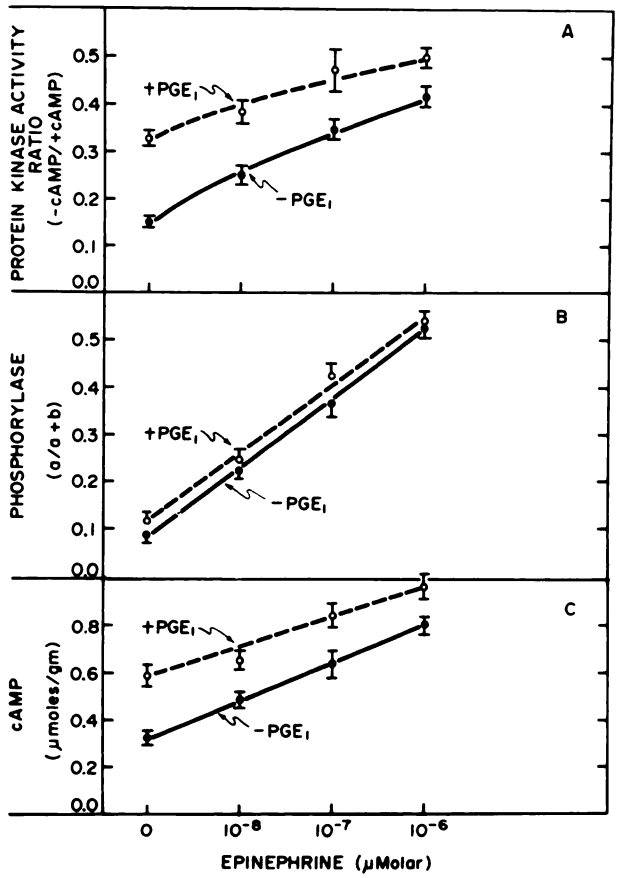

FIG. 3. *Effect of PGE_1 on epinephrine-induced increases in protein kinase (A), phosphorylase (B), and cAMP (C)*

Hearts were perfused for 15 min with buffer alone and then for an additional 2 min with the indicated concentration of epinephrine in the presence (○—○) or absence (●—●) of 5 μM PGE_1. Hearts were processed and assayed as described under EXPERIMENTAL PROCEDURES. Each point represents the mean ± SEM for 15 hearts.

tions 3 and 4). A similar observation has been made by Rodan and Feinstein working with platelets (23). This inhibition was presumably due to calcium influx inhibiting PGE_1 stimulation of adenylate cyclase. If higher concentrations of PGE_1 or PGE_1 in combination with 2-isobutyl-1-methylxanthine were used, protein kinase activities similar to those observed in the absence of A-23187 were obtained. Under no conditions was the increase in phosphorylase or force produced by PGE_1 plus ionophore similar to that seen with epinephrine.

Effect on phosphorylase kinase. As is shown in Table 4, 10 μM PGE_1, while producing a 80% increase in the cAMP-dependent protein kinase activity ratio, did not produce a detectable increase in the phosphorylase kinase ratio (pH 6.8/8.2). If higher cAMP-dependent protein kinase activation states were generated through the use of 100 μM PGE_1, or 10 μM PGE_1 in combination with isobutylmethylxanthine, a significant increase in phosphorylase kinase activity was observed. This increase was, however, always less than that seen when similar protein kinase activities were generated by epinephrine treatment. There appeared to be a slight increase in phosphorylase activity in hearts perfused with the higher PGE_1 concentration or PGE_1 plus isobutylmethylxanthine.

Effect of the presence of charcoal in homogenization medium. It was of concern

TABLE 1

Nonadditive Effects of Epinephrine plus PGE_1

Hearts were perfused for 15 min with buffer alone and then for an additional 2 min with buffer containing the indicated concentrations of prostaglandin E_1 (PGE_1), epinephrine (EPI), or 3-isobutyl-1-methylxanthine (IMX). Hearts were frozen and processed for assay as decribed under EXPERIMENTAL PROCEDURES. Each value represents the mean ± SEM of 4 to 8 hearts.

Perfusate additions	cAMP	Protein kinase activity ratio
	(nmoles/g)	*(−cAMP/ +cAMP)*
1) None	0.26 ± 0.02	0.19 ± 0.02
2) 30 μM PGE_1 + 1 μM IMX	0.92 ± 0.06	0.45 ± 0.02
3) 0.1 μM EPI + 1 μM IMX	1.96 ± 0.16	0.72 ± 0.06
4) 30 μM PGE_1 + 0.1 μM EPI + 1 μM IMX	1.84 ± 0.18	0.72 ± 0.07
5) 100 μM PGE_1	0.90 ± 0.10	0.43 ± 0.04
6) 0.1 μM EPI	0.70 ± 0.12	0.35 ± 0.03
7) 100 μM PGE_1 + 0.1 EPI	1.01 ± 0.07	0.51 ± 0.03
8) 100 μM EPI	1.92 ± 0.21	0.85 ± 0.11
9) 100 μM EPI + 100 μM PGE_1	189 ± 0.15	0.86 ± 0.12

that PGE_1 might elevate cAMP in a particular cell type not involved in the regulation of glycogen metabolism or contractile force and that release of this cAMP upon homogenization could activate protein kinase in the crude homogenate. To explore this possibility, experiments were conducted in which charcoal, which binds cAMP (6), was added to the homogenization buffer. As shown in Table 5, charcoal had little effect on either the basal or the PGE_1- or epinephrine-stimulated protein kinase activity ratio. The small decrease in the activity ratio could be due to a slight reassociation of the subunits in the absence of cAMP. In all cases, charcoal affected the PGE_1- and epinephrine-stimulated protein kinase activity ratios in the same manner.

DISCUSSION

Evidence from this and other laboratories has implicated the heart cyclic AMP-dependent protein kinase as one of the mediators of the actions of many cardioactive agents on both phosphorylase activation and contractile force (6–14). Results presented in this paper suggest that activation of the protein kinase in itself may not be sufficient to completely explain the action of these agents on either glycogen metabolism of contractility.

When concentrations of PGE_1 and epinephrine were adjusted to give equal increases in the cAMP-dependent protein kinase activity ratio (from a basal value of 0.15 to as high as 0.40),[2] only epinephrine produced a significant increase in contractile force or phosphorylase activity. If activity ratios greater than 0.40 were generated by treatment with very high concentrations of PGE_1 or PGE_1 in combination with a phosphodiesterase inhibitor, slight increases in phosphorylase activity were observed. These increases were, however, always much less than those seen when epinephrine was used to produce a similar protein kinase activation. The possibility that PGE_1 might have a dual effect—one to activate protein kinase and a second to block or reverse phosphorylase activation—is not likely since when both agents were added to the perfusion medium, PGE_1 did not attenuate the effect of epinephrine on phosphorylase activation.

The results reported here could be explained by postulating two distinct actions for epinephrine: (1) activation of protein kinase and (2) a second action, independent of the first, which allows protein kinase to act on the phosphorylase and contractile systems. PGE_1 presumably would not possess this second action. A cAMP-independent alteration in calcium movements would be an obvious choice for the second possible action of epinephrine. It should be noted that Namm *et al.* have observed that if calcium is removed from the perfusion medium, epinephrine will not activate phosphorylase in the perfused rat heart although it does elevate cAMP (24). In an effort to increase the intracellular calcium level in addition to activating protein kinase, hearts were treated with PGE_1 in the presence of α-adrenergic stimulation or ion-

[2] An activity ratio of 0.40 produces maximal increases in phosphorylase and contractile force (6, 9, 14).

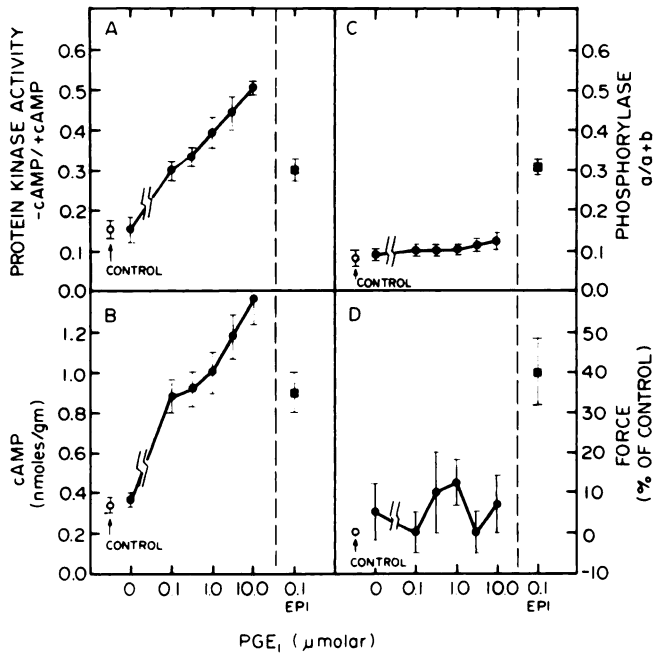

FIG. 4. *Effect of 3-isobutyl-1-methylxanthine on PGE_1-induced increases in protein kinase (A), cAMP (B), phosphorylase (C), and contractile force (D)*

Hearts were perfused for 15 min with buffer alone and an additional two minutes with buffer containing the indicated concentration of PGE_1 plus 1 μM epinephrine. Hearts were processed and assayed as described under EXPERIMENTAL PROCEDURES. Each point represents mean ± SEM of 10 hearts.

TABLE 2

Effect of Phenylephrine on PGE_1-Induced Increases in Protein Kinase, Phosphorylase, and cAMP

Hearts were perfused for 15 min with buffer alone and then for an additional 2 min with buffer containing the α-adrenergic agonist phenylephrine (PHEN), PGE_1, or epinephrine (EPI). Hearts were frozen and processed for assay as described under EXPERIMENTAL PROCEDURES. Each value represents the mean ± SEM of 5 hearts.

Additions	Protein kinase activity ratio	Phosphorylase a/a+b	cAMP
			(nM/g)
1) None	0.155 ± 0.06	0.080 ± 0.011	0.35 ± 0.02
2) 0.1 μM PHEN	0.172 ± 0.010	0.120 ± 0.022	0.32 ± 0.03
3) 5 μM PGE_1	0.421 ± 0.041[a]	0.105 ± 0.015	0.62 ± 0.05[a]
4) 5 μM PGE_1 + 0.1 μM PHEN	0.320 ± 0.035[a]	0.097 ± 0.013	0.54 ± 0.04[a]
5) 0.1 μM EPI	0.305 ± 0.022[a]	0.320 ± 0.042[a]	0.58 ± 0.04[a]

[a] Significantly different from control values at $p < 0.01$.

ophore A-23187. Both procedures can, under some circumstances, increase calcium influx (25, 26). Although there were indications that intracellular calcium was elevated, neither procedure altered the inability of PGE_1 to increase phosphorylase or force. While not completely excluding the possibility, these observations suggest that the initial reason for the differing actions of PGE_1 and epinephrine on phosphorylase activity and contractility might not be due entirely to differences in their abilities to alter calcium fluxes.

Another explanation would be that the two agents activate cAMP-dependent protein kinases located in different cell populations within the heart. Observations similar to this have been made in liver and adipose tissue (27, 28) and with the marked cell heterogeneity of the heart (29), this

TABLE 3

Effect of A-23187 on PGE_1-Induced Increases in Protein Kinase, Phosphorylase, cAMP, and Force

Hearts were perfused for 15 min with buffer alone and then for an additional 2 min with buffer containing prostaglandin E_1 (PGE_1), ionophore A-23187, 3-isobutyl-1-methylxanthine (IMX) or epinephrine (EPI). Hearts were frozen and processed for assay as described under EXPERIMENTAL PROCEDURES. Each value represents the mean ± SEM of 5 hearts.

Additions	Protein kinase activity ratio	Phosphorylase (a/a+b)	cAMP	Force (% of control)
			(nMoles/g)	
1) None	0.156 ± 0.009	0.065 ± 0.007	0.33 ± 0.33	100
2) 1.0 μM A-23187	0.149 ± 0.010	0.096 ± 0.012	0.30 ± 0.04	94 ± 8
3) 1.0 μM PGE_1	0.354 ± 0.017[a]	0.089 ± 0.015	0.69 ± 0.05[a]	95 ± 10
4) 1.0 μM PGE_1 + 1.0 μM A-23187	0.229 ± 0.017[a]	0.117 ± 0.042	0.50 ± 0.04[a]	102 ± 7
5) 50 μM PGE_1 + 10 μM A-23187	0.300 ± 0.020[a]	0.098 ± 0.017	0.68 ± 0.05[a]	110 ± 7
6) 10 μM PGE_1 + 1 μM IMX + 1 μM A-23187	0.349 ± 0.030[a]	0.089 ± 0.009	0.69 ± 0.02[a]	102 ± 10
7) 0.1 μM EPI	0.366 ± 0.025[a]	0.360 ± 0.035[a]	0.75 ± 0.06[a]	174 ± 14[a]

[a] Significantly different from control values at $p < 0.05$.

TABLE 4

Effect of Epinephrine and PGE_1 on Phosphorylase Kinase

Hearts were perfused for 15 min with buffer alone and then for an additional 2 min with buffer containing the indicated concentrations of prostaglandin E_1 (PGE_1), 3-isobutyl-1-methylxanthine (IMX) and/or epinephrine (EPI). Hearts were frozen and processed for assay as described under EXPERIMENTAL PROCEDURES. Each value represents the mean ± SEM of 6 to 21 hearts.

Perfusate addition	Protein kinase activity ratio −cAMP/+cAMP	Phosphorylase a/a+b	Phosphorylase kinase (6.8/8.2)
1) None	0.19 ± 0.01	0.10 ± 0.01	0.082 ± 0.009
2) 10 μM PGE_1	0.34 ± 0.04[a]	0.09 ± 0.01	0.079 ± 0.028
3) 100 μM PGE_1	0.44 ± 0.05[a]	0.19 ± 0.03[a]	0.131 ± 0.025[a]
4) 1 μM IMX	0.23 ± 0.02	0.15 ± 0.03	0.099 ± 0.011
6) 10 μM PGE_1 + 1 μM IMX	0.37 ± 0.03[a]	0.16 ± 0.03	0.119 ± 0.005[a]
7) 0.2 μM EPI	0.37 ± 0.04[a]	0.30 ± 0.03[a]	0.170 ± 0.016[a]

[a] Significantly different from control values of $p < 0.05$.

possibility must be considered. The fact that PGE_1 could not produce complete activation of the total heart protein kinase would support the idea that this agent does not act on all cell types in the perfused rat heart. The combined effects of PGE_1 and epinephrine were not additive when a maximum dose of the latter agent was used. Further, epinephrine can activate all the cAMP-dependent protein kinase in the perfused rat heart while PGE_1 cannot. Both these observations strengthen the evidence that the adrenergic agent is capable of increasing cAMP and protein kinase activity in the same cell type that PGE_1 does but that PGE_1 does not possess the same action in other cell types acted on by epinephrine. Thus all the results reported in the investigation could be explained by assuming that PGE_1 activates a cAMP-dependent protein kinase in a cell type that is not involved in the regulation of phosphorylase activity or contractile force.

An alternative explanation would be that while both PGE_1 and epinephrine might act on the same cell type that the two agents activate cAMP-dependent protein kinases located in different regions of these cells. We have recently shown that the heart contains at least two general types of

TABLE 5

Effect of Charcoal Addition to Homogenizing Medium on Protein Kinase Activation by PGE_1 and Epinephrine

Hearts were perfused, powdered and stored as described under EXPERIMENTAL PROCEDURES. Where indicated, PGE_1 (33 μM) plus 3-isobutyl-1-methylxanthine (1 μM) or epinephrine (0.5 μM) was added to the perfusion buffer (2 min). Homogenization (50 μl buffer/mg) was performed in 10 mM potassium phosphate buffer containing 10 mM EDTA and 0.5 mM 3-isobutyl-1-methylxanthine. In some cases charcoal was added to the buffer prior to homogenization, the homogenates were immediately centrifuged and assayed. Each value represents the mean ± SEM of 3 hearts.

Perfusate additions	Additions to homogenization medium	Protein kinase activity ratios
None	None	0.18 ± 3
"	1 mg/ml charcoal	0.15 ± 2
"	5 mg/ml charcoal	0.12 ± 5
PGE_1 + IMX	None	0.52 ± 4
"	1 mg/ml charcoal	0.51 ± 8
"	5 mg/ml charcoal	0.42 ± 4
Epinephrine	None	0.51 ± 2
"	1 mg/ml charcoal	0.41 ± 8
"	5 mg/ml charcoal	0.37 ± 3

cAMP-dependent protein kinases (type I and type II), as well as membrane associated and cytosolic forms of the enzymes (7, 30). It is likely that the different enzyme forms serve different cellular functions. It may be that PGE_1 activates a protein kinase which has little or no role in the regulation of phosphorylase activity or contractile force while epinephrine treatment produces an activation of this protein kinase, but also of the enzymes that are involved in the regulation of glycogen metabolism and contractile force. This possibility is currently being investigated.

REFERENCES

1. Sutherland, E. W., Robison, G. A., & Butcher, R. W. (1968) Some aspects of the biological role of adenosine 3',5'-monophosphate. *Circulation*, **37**, 279–306.
2. Robison, G. A., Butcher, R. W., & Sutherland, E. W. in *Cyclic AMP*. New York: Academic Press, 1971.
3. Mayer, S. E. (1972) Effects of adrenergic agonists and antagonists on adenylate cyclase activity of dog heart and liver. *J. Pharmacol. Exptl. Therap.*, **181**, 116–125.
4. Entman, M. L. (1974) The role of cyclic AMP in the modulation of cardiac contractility. *Adv. Cyclic Nucleotide Res.*, **4**, 163–193.
5. Kuo, J. F., & Greengard, P. (1969) Cyclic nucleotide-dependent protein kinases. IV. Widespread occurrence of adenosine 3',5'-monophosphate-dependent protein kinase in various tissues and phyla of the animal kingdom. *Proc. Natl. Acad. Sci. USA*, **64**, 1349–1355.
6. Keely, S. L., Corbin, J. D., & Park, C. R. (1975) Regulation of adenosine 3':5'-monophosphate-dependent protein kinase. *J. Biol. Chem.*, **250**, 4832–4840.
7. Corbin, J. D., & Keely, S. L. (1977) Characterization and regulation of heart adenosine 3':5'-monophosphate-dependent protein kinase isozymes. *J. Biol. Chem.*, **252**, 910–918.
8. Keely, S. L., Corbin, J. D., & Lincoln, T. (1977) Alpha adrenergic involvement in heart metabolism: Effects on adenosine cyclic 3',5'-monophosphate, adenosine cyclic 3',5'-monophosphate-dependent protein kinase, guanosine cyclic 3',5'-monophosphate, and glucose transport. *Mol. Pharmacol.*, **13**, 965–975.
9. Keely, S. L., & Corbin, J. D. (1977) Involvement of cAMP-dependent protein kinase in the regulation of heart contractile force. *Am. J. Physiol.*, **233**, H269–H275.
10. Kirchberger, M. A., Tada, M. & Katz, A. M. (1974) Adenosine 3':5'-monophosphate-dependent protein kinase-catalyzed phosphorylation reaction and its relationship to calcium transport in cardiac sarcoplasmic reticulum. *J. Biol. Chem.*, **249**, 6166–6173.
11. Morkin, E., & LaRaia, P. J. (1974) Biochemical studies on the regulation of myocardial contractility. *N. Engl. J. Med.*, **290**, 445–451.
12. Dobson, J. G. (1978) Protein kinase regulation of cardiac phosphorylase activity and contractility. *Am. J. Physiol.*, **234**, H638–H645.
13. Jesmok, G. J., Calvert, D. N., & Lech, J. J. (1977) The effect of inotropic agents on glycerol release and protein kinase activity ratios in the isolated perfused rat heart. *J. Pharmacol. Exp. Ther.*, **200**, 187–194.
14. Keely, S. L., & Ering, A. F. Involvement of cAMP-dependent protein kinase in the regulation of heart contractile force. *Am. J. Physiol.* (In press).
15. Keely, S. L., Corbin, J. D. & Park, C. R. (1975) On the question of translocation of heart cAMP-dependent protein kinase. *Proc. Natl. Acad. Sci. USA*, **72**, 1501–1504.
16. Gilman, A. G. (1970) A protein binding assay for adenosine 3':5'-cyclic monophosphate. *Proc. Natl. Acad. Sci. USA*, **67**, 305–312.
17. Gilboe, D. P., Larson, K. L. & Nuttall, F. Q. (1972) Radioactive method for the assay of glycogen phosphorylases. *Anal. Biochem.*, **47**, 20–27.

18. Krebs, E. G., Love, D. S., Bratvold, G. E., Trayser, K. A., Meyer, W. L., & Fischer, E. H. (1964) Purification and properties of rabbit skeletal muscle phosphorylase *b* kinase. *Biochemistry*, **3**, 1022–1033.
19. Drummond, G. I. & Duncan, L. (1966) Activation of cardiac phosphorylase *b* kinase. *J. Biol. Chem.*, **241**, 5893–5898.
20. Glynn, I. M. & Chappell, J. B. (1964) A simple method for the preparation of ^{32}P-labelled ATP of high specific activity. *Biochem. J.*, **90**, 147–149.
21. Walsh, D. A., Ashby, C. D., Gonzalez, C., Calkins, D., Fischer, E. H. & Krebs, E. G. (1971) Purification and characterization of a protein inhibitor of adenosine 3'5'-monophosphate-dependent protein kinases. *J. Biol. Chem.*, **246**, 1977–1985.
22. Watanabe, A. M., Hathaway, D. R., Besch, H. R., Farmer, B. B. & Harris, R. A. (1977) Alpha adrenergic reduction of cyclic adenosine monophosphate concentrations in rat myocardium. *Circ. Res.*, **40**, 596–602.
23. Rodan, G. A., & Feinstein, M. B. (1976) Interrelationships between Ca^{++} and adenylate and guanylate cyclases in the control of platelet secretion and aggregation. *Proc. Natl. Acad. Sci. USA*, **73**, 1829–1833.
24. Namm, D. H., Mayer, S. E. & Maltbie, M. (1968) The role of potassium and calcium ions in the effect of epinephrine on cardiac cyclic adenosine 3',5'-monophosphate, phosphorylase kinase, and phosphorylase. *Mol. Pharmacol.*, **4**, 522–530.
25. Assimacopoulos-Jeannet, F. D., Blackmore, P. F. & Exton, J. H. (1977) Studies on alpha-adrenergic activation of hepatic glucose output. *J. Biol. Chem.*, **252**, 2662–2669.
26. Hainaut, K., & Desmedt, J. E. (1974) Calcium ionophore A23187 potentiates twitch and intracellular calcium release in single muscle fibres. *Nature*, **252**, 407–408.
27. Ferretti, E., Biondi, C. & Tomasi, V. (1976) Cyclic AMP control by prostaglandin E_1 in non-parenchymal liver cells. *FEBS Letters*, **69**, 70–74.
28. Butcher, R. W., Baird, C. E. & Sutherland, E. W. (1968) Effect of lipolytic and antilipolytic substances on adenosine 3',5'-monophosphate levels in isolated fat cells. *J. Biol. Chem.*, **243**, 1705–1712.
29. Grove, D., Zak, R., Nair, K. G. & Aschenbrenner, V. (1969) Biochemical correlates of cardiac hypertrophy. *Circ. Res.*, **25**, 473–485.
30. Corbin, J. D., Sugden, P. H., Lincoln, T. M. & Keely, S. L. (1977) Compartmentalization of adenosine 3':5'-monophosphate and adenosine 3':5'-monophosphate-dependent protein kinase in heart tissue. *J. Biol. Chem.*, **252**, 3854–3861.