MOLECULAR PHARMACOLOGY, 13, 965-975

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

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(Received March 8, 1977)
(Accepted May 26, 1977)

SUMMARY

KEELY, STANLEY L., CORBIN, JACKIE D. & LINCOLN, THOMAS (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.

In the perfused rat heart the *alpha* adrenergic agonist phenylephrine, at concentrations of 0.1-50 μM, increased the adenosine cyclic 3',5'-monophosphate (cAMP)-dependent protein kinase activity ratio in a dose-dependent manner. In all cases there was a corresponding increase in glycogen phosphorylase activity. When phenylephrine concentrations greater than 1 μM were used, there was a significant increase in cAMP associated with the rise in protein kinase activity; however, the modest increase in kinase activity produced by concentrations less than 1 μM was not accompanied by any detectable increase in cAMP. The *beta* adrenergic blocking agent propranolol did not inhibit activation of the cAMP-dependent protein kinase in hearts treated with low concentrations (1 μM or less) of phenylephrine, but did reduce the greater activation seen in hearts treated with higher concentrations (5 μM or more) of the drug. Phentolamine (1 μM), an *alpha* adrenergic blocking agent, was found to potentiate the effect of phenylephrine concentrations greater than 5 μM. When animals were treated with reserpine 18 hr prior to death, activation of cAMP-dependent protein kinase by phenylephrine was still evident. The phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine potentiated the effect of phenylephrine on all parameters measured. Calcium was necessary for the activation of protein kinase by low (1 μM or less) but not high (5 μM or greater) concentrations of the drug. Although 1 μM phenylephrine produced a slight activation of heart protein kinase when used alone, it reduced the ability of *beta* adrenergic agonists (e.g., isoproterenol) to activate the enzyme. Epinephrine, which has both *alpha* and *beta* adrenergic components, was a more effective activator of heart cAMP-dependent protein kinase when its *alpha* component was blocked with phentolamine than when epinephrine was used alone. These observations indicate that kinase activation is principally through a *beta* adrenergic mechanism. Epinephrine also increased glucose transport by a process which was blocked by phentolamine but not

This work was supported by Research Grant HL 19181, Program Project Grant AM 07462, Diabetes Endocrinology Center Grant AM 17026 from the United States Public Health Service and Research Grant AM 15988.

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ISSN 0026-895X

propranolol. Phenylephrine, in the presence of 3-isobutyl-1-methylxanthine, significantly increased guanosine cyclic 3′,5′-monophosphate levels. This increase was blocked by the *alpha* antagonist phentolamine but not by the *beta* antagonist propranolol.

INTRODUCTION

It is well established that adenosine cyclic 3′,5′-monophosphate, either alone or in combination with other factors, mediates many of the effects of *beta* adrenergic agents on cardiac glycogenolysis, lipolysis, and contractility (1–7). It has been suggested that many of the effects of cAMP[3] are mediated by changes in the activity of cAMP-dependent protein kinases (8). Recent work from our laboratory has shown that in the perfused rat heart, epinephrine will produce activation of these enzymes (9, 10). Entman *et al.* (11), Morkin and LaRaia (12), and Kirchberger *et al.* (13) have shown that cAMP and the cAMP-dependent protein kinase will stimulate calcium uptake by partially purified sarcoplasmic reticulum from cardiac tissue. Katz and Repke (14) have suggested that the positive inotropic effect of epinephrine might be due to a protein kinase-induced increase in calcium uptake by the sarcoplasmic reticulum. Wray *et al.* (15) have shown that an endogenous cAMP-dependent protein kinase will catalyze phosphorylation of cardiac sarcoplasmic reticulum.

Grover (16), Benfey and Carolin (17), Hamakawa *et al.* (18), Endoh *et al.* (19), and Osnes (20) have reported that *alpha* adrenergic stimulation of cardiac muscle produces a positive inotropic response. In cases when cAMP levels were measured, no increase in the cyclic nucleotide levels were detected (17, 20), although in other experiments McNeill and Verma (21) have reported that large doses (more than 0.4 mg/kg) of the *alpha* adrenergic agonist phenylephrine produced a significant increase in heart cAMP levels.

To determine whether the effect of *alpha* adrenergic stimulation might be mediated through cAMP-dependent protein kinase, either dependent on or independent of changes in cAMP, we have investigated the ability of phenylephrine to alter cAMP levels, to activate the cAMP-dependent protein kinase, and to increase glycogen phosphorylase activity. *Alpha* adrenergic effects on *beta* adrenergic activation of the cAMP-dependent protein kinase, cardiac cGMP level, and glucose transport were also investigated.

[3] The abbreviations used are: cAMP, adenosine cyclic 3′,5′-monophosphate; cGMP, guanosine cyclic 3′,5′-monophosphate.

EXPERIMENTAL PROCEDURE

Heart perfusion. Fed male rats weighing 100–150 g were used. Sodium heparin (1500 units/kg) was injected intraperitoneally 30 min before death. The animals were anesthetized with sodium pentobarbital intraperitoneally (100 mg/kg). The hearts were quickly excised, immersed in ice-cold NaCl solution until beating ceased, and attached via the aorta to the perfusion cannula. Perfusion was carried out by the Langendorff method at 37° with recirculation after an initial 3-min washout period at an aortic pressure of 60 mm Hg with Krebs-Henseleit bicarbonate buffer equilibrated with 95% O_2–5% CO_2 (22). The buffer contained 5 mM glucose and 1 mg/ml of bovine serum albumin. At the end of the perfusion period, the hearts were quickly frozen between two aluminum blocks which had been cooled in liquid nitrogen, and pulverized to a fine powder in a percussion mortar which had also been cooled in liquid nitrogen (23). The powder was stored at −70° until assayed. There was no loss in enzyme activity or change in activation state for either protein kinase or phosphorylase, or in the cAMP levels, even after several weeks of storage.

Protein kinase assay. Approximately 50 mg of powdered heart tissue were suspended at 4° in 15 times its weight of 10 mM potassium phosphate buffer (pH 6.8) containing 10 mM EDTA and 0.5 mM 3-isobutyl-1-methylxanthine, and homogenized in a glass homogenizer with a loosely fitting Teflon pestle. The homogenate was

centrifuged at 27,000 × *g* for 20 min at 4°. The protein kinase activity was determined by measuring the transfer of ^{32}P from [γ-^{32}P]ATP into histone in the presence and absence of added cAMP as described by Keely *et al.* (9). The assay reaction 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 (approximately 33 cpm/pmole), 6 mM magnesium acetate, 0.5 mg of histone, and 2 μM cAMP where indicated. The reaction was terminated by pipetting 50 μl of the reaction mixture onto a 1 × 2 cm filter paper disc, which was immediately dropped into cold 10% trichloroacetic acid (10 ml/disc). The filter paper was washed four times in trichloroacetic acid and once each in 95% ethanol and diethyl ether and dried. The radioactivity on each disc was measured in 10 ml of toluene–Cellosolve scintillation fluid. Protein kinase activity is expressed as an activity ratio, i.e., the ratio of histone kinase activity in the absence of added cAMP to that in the presence of 2 μM exogenous cAMP.

Cyclic AMP assay. Cyclic AMP was determined by the protein binding assay of Gilman (24) with minor modifications by Keely *et al.* (9).

Cyclic GMP assay. Cyclic GMP levels were determined by measuring binding to a partially purified rat lung cGMP-specific binding protein (cGMP-dependent protein kinase). Details of this assay are described elsewhere.[4]

Glucose transport. Glucose transport was determined by measuring efflux of labeled sugar from the heart. Hearts were perfused for 15 min as described above, and then for an additional 5 min with 10 ml of buffer containing 5 μCi of [^{14}C]3-*O*-methylglucose (50 μCi/μmole) plus 0.2 mg of nonradioactive methylglucose. The perfusion buffer was then changed to one free of radioactive 3-*O*-methylglucose, and the first 4 ml which passed through the heart, containing most of the extracellular label, were discarded. Then either epinephrine (0.5 μM), epinephrine (0.5 μM) plus propranolol (0.5 μM), epinephrine (0.5 μM) plus phentolamine (0.5 μM), or nothing (control) was added to the perfusion buffer. Flow through the heart was kept constant at 10 ml/min by means of a Harvard peristaltic pump. Perfusate flowing from the heart was collected in 2-ml fractions and counted in toluene–Triton X-100 (2:1) scintillation fluid.

Other methods. Phosphorylase activity was determined by measuring phosphorylase activity in the absence and presence of 0.3 mM 5′-AMP according to the method of Gilboe *et al.* (25). The cAMP-dependent protein kinase inhibitor was prepared by the method of Walsh *et al.* (26) through the trichloroacetic acid precipitation step.

Materials. Type II-A histone from calf thymus, fraction V bovine serum albumin, cAMP, and phenylephrine were obtained from Sigma. [γ-^{32}P]ATP was prepared by the method of Glynn and Chappell (27); 3-isobutyl-1-methylxanthine was a gift from Searle and Company; and reserpine was obtained from Ciba.

RESULTS

Phenylephrine concentration dependence. Figure 1 shows the cAMP level, protein kinase activity ratio, and phosphorylase activity ratio in response to increasing concentrations of phenylephrine. Assays were performed on tissue extracts from hearts which had been perfused for 3 min with constant infusion of the adrenergic agent. Significant activation of the protein kinase occurred at phenylephrine concentrations of 0.5 μM and higher. There was, however, no detectable increase in cAMP until the phenylephrine concentration exceeded 1 μM. At all concentrations tested, increases in protein kinase activity were accompanied by similar increases in phosphorylase activity. As shown in Table 1, the increase in protein kinase activity was completely inhibited by the cAMP-dependent protein kinase inhibitor. Basal protein kinase activity was also reduced by the inhibitor. Since the protein inhibitor acts exclusively on the free catalytic subunit (28), this would suggest that the basal protein kinase activity is due primarily to

[4] S. L. Keely, J. D. Corbin, and T. L. Lincoln, manuscript submitted for publication.

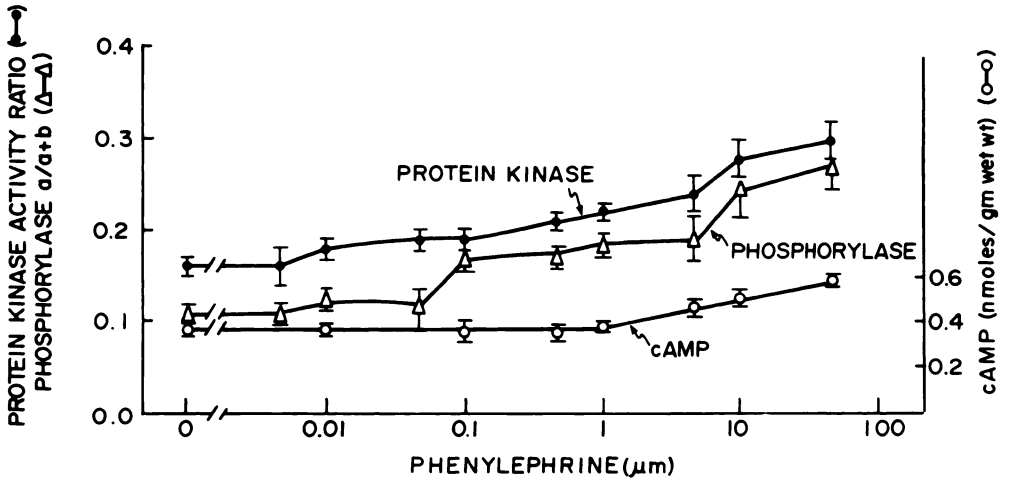

FIG. 1. *Effect of phenylephrine concentration on cAMP levels, protein kinase activity ratio, and phosphorylase a/(a+b) in perfused rat heart*

Hearts were first perfused for 15 min as described and then for an additional 3 min with buffer containing the indicated concentration of phenylephrine. Handling of the tissue for assay is described under EXPERIMENTAL PROCEDURE. Each point represents the mean ± standard error of 15 hearts.

TABLE 1

Effect of cAMP-dependent protein kinase inhibitor protein on phenylephrine-induced elevation of protein kinase activity

Hearts were perfused for 15 min with buffer alone and then for an additional 3 min with buffer containing 1 or 10.0 μM phenylephrine. Hearts were processed for assay as described under EXPERIMENTAL PROCEDURE. Protein kinase activity was determined in the absence and presence of 2 μM cAMP and in the absence and presence of 20 units of protein kinase inhibitor protein [1 unit of inhibitor is the amount necessary to block 1 unit of purified heart type II cAMP-dependent protein kinase (10)].

Phenylephrine	Total protein kinase activity			
	−Inhibitor		+Inhibitor	
	−cAMP	+cAMP	−cAMP	+cAMP
μM	*units/mg protein*			
0	280 ± 25	1860 ± 70	60 ± 10	122 ± 47
1	350 ± 20	1900 ± 55	65 ± 55	133 ± 14
10	420 ± 25	1820 ± 60	65 ± 10	150 ± 20

dissociation of the enzyme and not to residual activity of the holoenzyme.

Time course of phenylephrine activation. When hearts were perfused with 1 μM phenylephrine, at least 15 sec were required for an increase in the protein kinase activity ratio to be detected, and more than 1 min was needed for maximal activation of the enzyme (Fig. 2). Phosphorylase reached maximal activity at approximately 2 min, after which it began to decline even though the protein kinase activity ratio remained elevated. At no time from 15 sec to 5 min was there a significant increase in the cAMP levels.

When hearts were treated with 10 μM phenylephrine (dashed lines), the time course for protein kinase and phosphorylase activation was similar to, although of greater magnitude than, the curves obtained with a 1 μM concentration. With higher phenylephrine concentration there was a significant increase in cAMP, which correlated well with protein kinase activation.

Effects of blocking agents on phenylephrine activation of protein kinase. Phenylephrine (1 μM) raised the cAMP-dependent protein kinase activity ratio from a control value of 0.13 to a stimulated level of 0.18 (Fig. 3). Neither the *alpha* adrenergic antagonist phentolamine (1 μM) nor the *beta* adrenergic blocker propranolol (1 μM) reduced this modest increase in protein kinase activity. Phenylephrine at 10 μM produced a greater increase in the kinase activity ratio (0.23), which was potentiated by the *alpha* blocking agent phentolamine. The effect of the higher concentration of phenylephrine on the activity

ratio was diminished by propranolol to a level similar to that obtained with 1 μM phenylephrine. All changes in protein kinase activity paralleled changes in the cAMP levels except for hearts treated with 1 μM phenylephrine, which exhibited no significant change in cAMP.

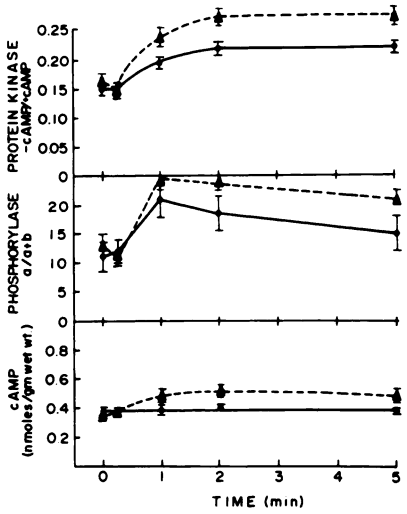

FIG. 2. *Time course of effect of 1 μM (●——●) or 10 μM (▲- - -▲) phenylephrine on protein kinase activity ratio, phosphorylase a/(a+b), and cAMP level*

Hearts were perfused as described in Fig. 1 with buffer alone and then for the indicated time with buffer containing 1 μM or 10 μM phenylephrine. Each point represents the mean ± standard error of 14 hearts.

Effect of reserpine treatment. To determine whether the phenylephrine-induced activation of protein kinase might be due to release of endogenous norepinephrine stores, the effect of reserpine treatment on protein kinase activation was investigated. Rats were injected intraperitoneally with reserpine (5 mg/kg) 18 hr prior to death. Hearts were removed and perfused as described under EXPERIMENTAL PROCEDURE with either 1 μM or 10 μM phenylephrine. As shown in Fig. 4, treatment with reserpine slightly reduced basal protein kinase activity (compare with Figs. 1–3) but did not block the ability of phenylephrine to activate protein kinase. Again, no increase in cAMP levels was seen with the lower dose of phenylephrine, although a significant increase was obtained when the agent was infused at a 10 μM concentration.

Effect of 3-isobutyl-1-methylxanthine. The phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine potentiated the effect of phenylephrine on protein kinase activity (Table 2). A similar effect was seen on phosphorylase activity. Although neither agent alone produced a detectable

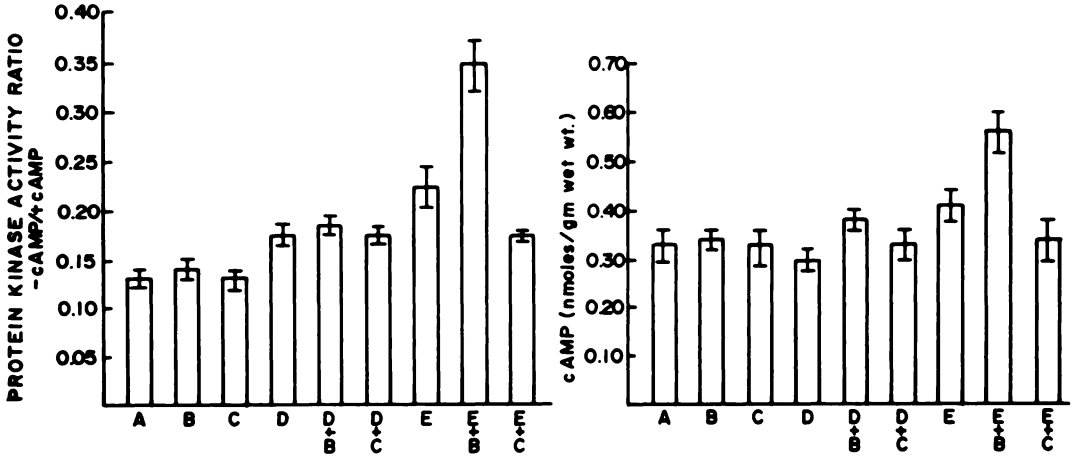

FIG. 3. *Effect of adrenergic blocking agents on phenylephrine activation of heart protein kinase*

Hearts were perfused for 15 min with Krebs-Henseleit bicarbonate buffer alone and then for an additional 3 min with buffer containing either no additions (A), 1 μM phentolamine (B), 1 μM propranolol (C), 1 μM phenylephrine (D), or 10 μM phenylephrine (E). Hearts were processed as described under EXPERIMENTAL PROCEDURE. Each bar represents the mean ± standard error of 15 hearts.

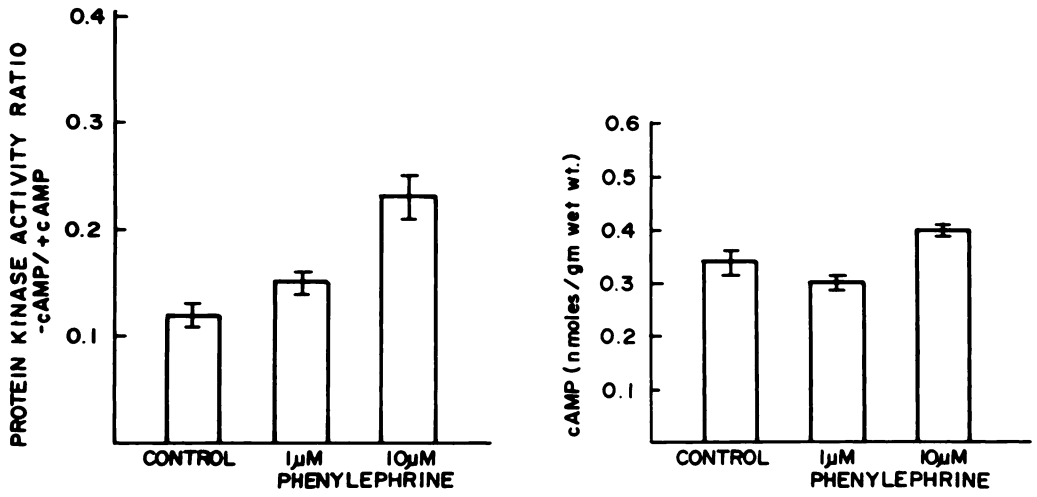

FIG. 4. *Phenylephrine activation of cAMP-dependent protein kinase in hearts from rats treated with reserpine*

Rats were injected intraperitoneally 18 hr prior to death with reserpine (5 mg/kg). Hearts were removed from the animals as described under EXPERIMENTAL PROCEDURE and perfused for 15 min with buffer alone, followed by a 3-min perfusion with either 1 μM or 10 μM phenylephrine. Each bar represents the mean ± standard error of eight hearts.

TABLE 2

Effect of 3-isobutyl-1-methylxanthine (IBMX) on phenylephrine (PHEN) activation of heart protein kinase, phosphorylase, and cAMP levels

Hearts were perfused for 15 min with buffer alone and then for an additional 3 min with buffer containing the indicated drugs. Hearts were processed and assayed as described under EXPERIMENTAL PROCEDURE. Each value represents the mean ± standard error of 12 hearts.

Drug	Protein kinase activity ratio	cAMP	Phosphorylase *a*/(*a*+*b*)
		nmole/g, wet wt.	
None	0.14 ± 0.01	0.37 ± 0.02	0.10 ± 0.02
1 μM IBMX	0.16 ± 0.02	0.40 ± 0.02	0.15 ± 0.02
0.5 μM PHEN	0.18 ± 0.01	0.38 ± 0.01	0.17 ± 0.02
0.5 μM PHEN + 1 μM IBMX	0.22 ± 0.01	0.49 ± 0.02	0.25 ± 0.03
50 μM PHEN	0.28 ± 0.02	0.55 ± 0.04	0.24 ± 0.02
50 μM PHEN + 1 μM IBMX	0.41 ± 0.01	0.67 ± 0.05	0.46 ± 0.03

increase in cAMP, 3-isobutyl-1-methylxanthine, when used with 0.5 μM phenylephrine, did produce a significant increase in the cyclic nucleotide.

Effect of Ca^{++} on phenylephrine activation of protein kinase. When hearts were perfused with buffer containing 2% of the normal Ca^{++} concentration, the increase in the protein kinase activity ratio produced by 1 μM phenylephrine was eliminated while the kinase activation observed with 10 μM phenylephrine was not altered (Fig. 5). Muscular contractions ceased when the Ca^{++} concentration was reduced to 2% of the normal level. Flow through the heart was maintained by increasing the perfusion pressure.

Alpha adrenergic inhibition of isoproterenol-induced activation of protein kinase. Experiments described in Fig. 3 showed that 10 μM phenylephrine produced an activation of the cAMP-dependent protein kinase which was due to a *beta* adrenergic process (propranolol greatly reduced the amount of activation). Protein kinase activation could be potentiated if the *alpha* adrenergic activity of phenylephrine, its principal component, was

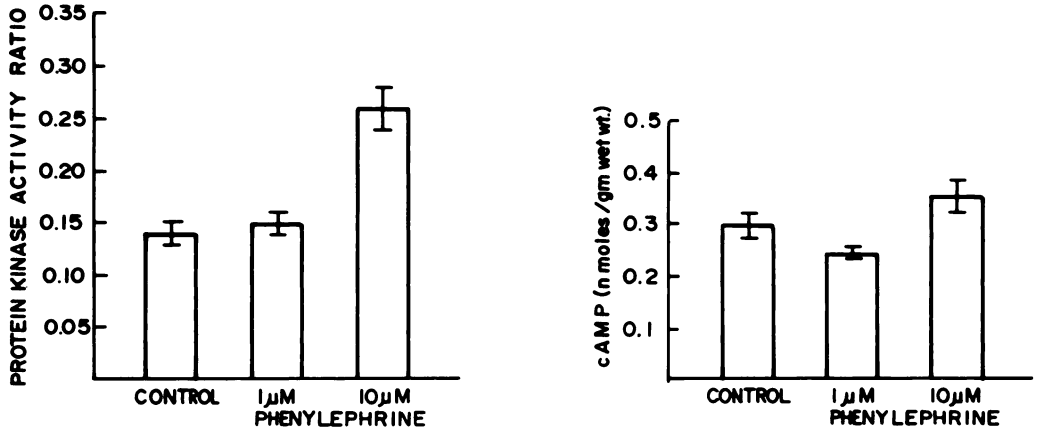

FIG. 5. *Effect of calcium concentration on phenylephrine activation of heart protein kinase*

Hearts were perfused for 15 min with buffer containing normal calcium levels (2.54 mM) and then for 15 min with buffer containing 2% of the normal Ca^{++} concentration. Hearts were then perfused for 3 min with the low-Ca^{++} buffer containing 1 μM or 10 μM phenylephrine. Each bar represents the mean ± standard error of 10 hearts.

blocked by phentolamine. This suggested that although *alpha* adrenergic stimulation might produce a slight activation of protein kinase, it would also substantially inhibit *beta* adrenergic activation of the enzyme. Figure 6 shows the effects of the *beta* adrenergic agonist isoproterenol, either alone or in combination with 5 μM phenylephrine, on cAMP-dependent protein kinase activity. Phenylephrine reduced the effect of isoproterenol at all concentrations tested. Figure 7 shows the effect of epinephrine, either alone or in combination with phentolamine or propranolol, on activation of protein kinase and glycogen phosphorylase. Propranolol greatly reduced the epinephrine-induced increase in both protein kinase and phosphorylase activities, while phentolamine enhanced the epinephrine-induced increases in both enzymes. Neither propranolol nor phentolamine had an effect on protein kinase or phosphorylase when used alone (data not shown).

Effect of phenylephrine on cardiac cGMP levels. It has recently been reported that *alpha* adrenergic stimulation of rat atrial muscle increased cGMP levels (29). We have investigated the effect of phenylephrine on cGMP in the perfused rat heart. When phenylephrine (10 μM) and 3-isobutyl-1-methylxanthine (2 μM) were added to the perfusion buffer, there was a

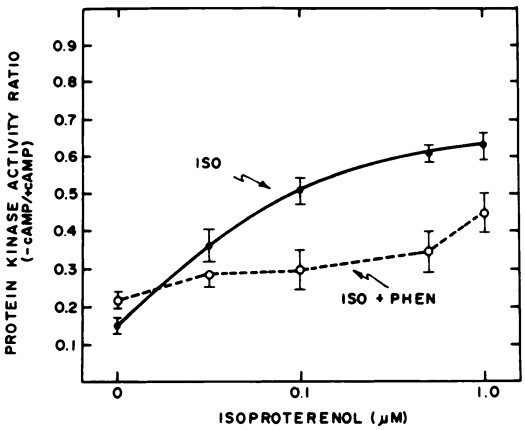

FIG. 6. *Effect of phenylephrine on isoproterenol activation of heart protein kinase*

Hearts were first perfused for 15 min with buffer alone and then for 3 min with buffer containing either isoproterenol (ISO) at the indicated concentration (●——●) or isoproterenol plus 5 μM phenylephrine (PHEN) (O- - -O). Hearts were freeze-clamped, processed, and assayed as described under EXPERIMENTAL PROCEDURE. Each point represents the mean ± standard error of four hearts.

2-fold increase in cGMP (Fig. 8). This increase was due to activation of *alpha* receptors in the heart, since phentolamine, but not propranolol, completely blocked the effect.

Effect of alpha adrenergic stimulation on glucose transport. It is well known that under some circumstances adrenergic

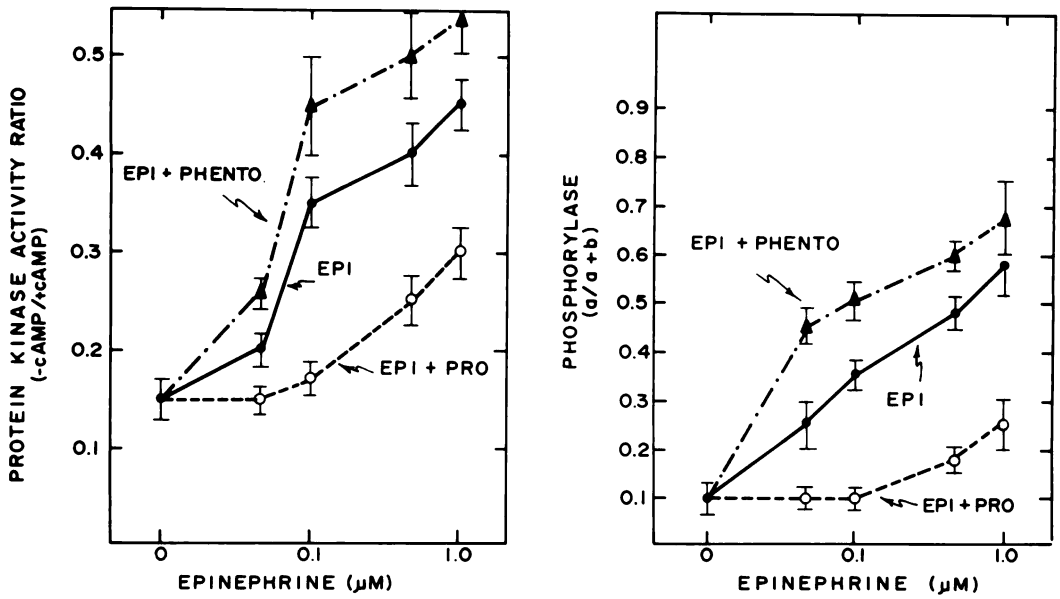

FIG. 7. *Effects of alpha and beta adrenergic antagonists on epinephrine activation of heart protein kinase*
Hearts were perfused for 15 min with buffer alone and then for 2 min with buffer containing the indicated concentration of epinephrine (EPI) (●——●), epinephrine plus 1 μM phentolamine (PHENTO) (▲-·-·▲), or epinephrine plus 1 μM propranolol (PRO) (O- - -O). Hearts were processed as described under EXPERIMENTAL PROCEDURE. Each point represents the mean ± standard error of three hearts.

agents can stimulate glucose transport. In some tissues this effect has been shown to be *alpha* in nature (30, 31). An investigation of the effect of adrenergic stimulation on glucose transport in the perfused heart suggests that it too is mediated by an *alpha* adrenergic process. Hearts were labeled with [^{14}C]3-*O*-methylglucose, and glucose transport was determined by measuring the rate of label washout. Either epinephrine, epinephrine plus phentolamine, or epinephrine plus propranolol was then added to the perfusion buffer; 22 ml of buffer washed through the heart and were collected in 2-ml fractions. Flow through the heart was 10 ml/min. Figure 9, upper left, shows the rate of 3-*O*-methylglucose efflux when no drugs were added to the perfusion buffer. The upper right-hand panel shows the rate of sugar efflux when epinephrine was added to the washout buffer. The peak in the washout curve indicates a stimulation in efflux. As shown at lower left, phentolamine completely blocked the epinephrine stimulation of transport, whereas propranolol (lower right) had no effect.

DISCUSSION

The ability of various adrenergic agents to raise heart cAMP levels has been well documented. The *beta* component of these drugs is thought to be responsible for this increase. It is generally accepted that many, if not all, of the effects of cAMP in mammalian systems are mediated by changes in the activation state of cAMP-dependent protein kinases. Recent work in this laboratory has demonstrated a positive correlation, in both time and degree, between the ability of epinephrine to activate heart cAMP-dependent protein kinase and its effect on contractile force (32). This work would implicate protein kinase as a mediator of the action of epinephrine on cardiac contractility. It has recently been demonstrated that the *alpha* adrenergic agonist phenylephrine will increase the force of contraction in various heart preparations (16–20). The inotropic effect of concentrations below 5 μM was blocked by *alpha* but not by *beta* adrenergic antagonists, while the reverse was true for phenylephrine concentrations greater than 5 μM (16). It has also been shown that

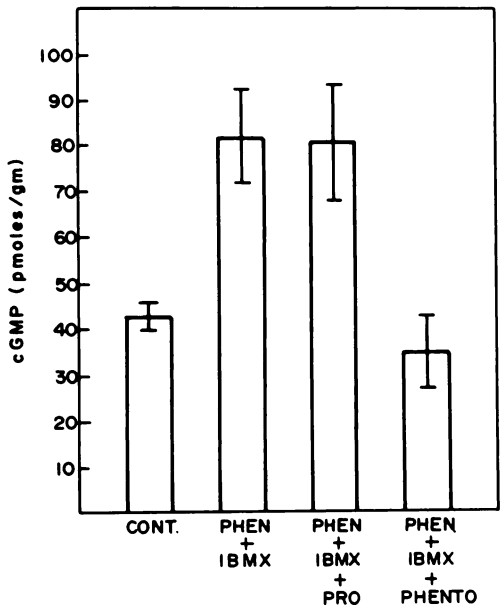

FIG. 8. *Effect of phenylephrine on cGMP levels in perfused rat heart*

Hearts were perfused for 15 min with buffer alone and then for 3 min with buffer containing (a) no additions (CONT.), (b) 50 μM phenylephrine (PHEN) plus 2 μM 3-isobutyl-1-methylxanthine (IBMX), (c) same as (b) plus 50 μM propranolol (PRO), or (d) same as (b) plus 50 μM phentolamine (PHENTO). Hearts were processed and assayed as described under EXPERIMENTAL PROCEDURE. Each bar represents the mean ± standard error of nine hearts.

low concentrations of phenylephrine (less than 5 μM) do not activate adenylate cyclase in crude heart homogenates (17), suggesting that the positive inotropic effect seen at concentrations less than 5 μM is not due to increased cAMP.

In this paper we have shown that in the perfused rat heart phenylephrine activates cAMP-dependent protein kinase and increases phosphorylase *a* levels. With concentrations below 1 μM and in the absence of phosphodiesterase inhibitors, activation of protein kinase and phosphorylase was not associated with any detectable increase in cAMP, although there was a significant increase in the cyclic nucleotide level with phenylephrine concentrations greater than 5 μM. Neither the *alpha* adrenergic blocking agent phentolamine nor the *beta* blocking agent propranolol prevented the activation of protein kinase by levels of phenylephrine which caused no detectable rise in cAMP. This observation suggests that the small activation of the protein kinase seen with concentrations of phenylephrine below 1 μM is not responsible for the inotropic effect observed with similar drug concentrations as reported by Grovier (16), Hamakawa *et al.* (18), Endoh *et al.* (19), and Osnes (20), since the contractile effects could be blocked by *alpha* adrenergic blocking agents while the effect on protein kinase activity could not be attenuated. Activation of protein kinase by phenylephrine concentrations which led to an increase in cAMP could be partially reduced by propranolol. Phentolamine did not reduce, but rather potentiated, the effect of phenylephrine concentrations which increase cAMP.

Robison *et al.* (33) have proposed that the divergent effects of *alpha* and *beta* adrenergic agents are due to opposing effects

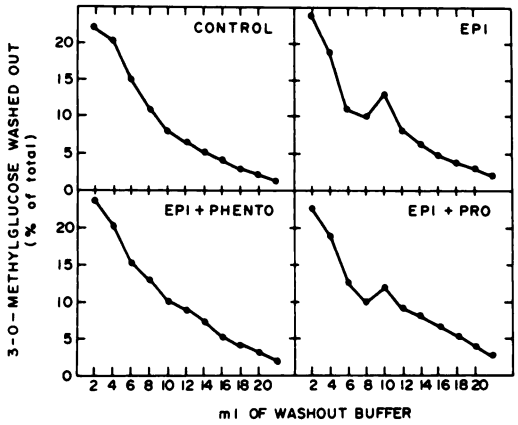

FIG. 9. *Effect of epinephrine on sugar transport in perfused heart*

Hearts were first perfused with buffer containing [^{14}C]3-*O*-methylglucose to label the intracellular glucose pools. Transport was determined by measuring the efflux of labeled sugar from the perfused heart. The first 4 ml, which contained most of the extracellular label, were discarded. The following additions were then made to the chase buffer: (a) none (control), (b) epinephrine (EPI) (0.5 μM), (c) epinephrine plus 0.5 μM phentolamine (PHENTO), or (d) epinephrine plus 0.5 μM propranolol (PRO). Transport is expressed as a percentage of the total amount of label collected appearing in each fraction. Each point represents the mean of five determinations.

on cAMP levels. Our findings support this hypothesis. Phenylephrine appears to have slight *beta* adrenergic activity, which becomes evident at concentrations greater than 1 μM, as indicated by the increases in cAMP and cAMP-dependent protein kinase. This effect on the *beta* adrenergic receptor would be antagonized by the action of phenylephrine on the *alpha* receptor. When *alpha* adrenergic stimulation was blocked with phentolamine, the full *beta* effect of the adrenergic agent was observed, with larger increases in both cAMP and protein kinase activity. The finding that phenylephrine will reduce the ability of isoproterenol, a relatively pure *beta* agonist, to activate heart protein kinase also supports the hypothesis of Robison *et al.*

Since phenylephrine caused activation of protein kinase in animals whose endogenous catecholamine stores had been depleted by treatment with reserpine, the action of phenylephrine is apparently direct and not due to release of endogenous norepinephrine. Whether the effect of phenylephrine on heart protein kinase is due to the production of a small or localized increase in cAMP, or to a cAMP-independent mechanism related to *alpha* adrenergic stimulation of heart muscle, is not known. When the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine was added to perfusion buffer together with a low concentration of phenylephrine (0.5 μM), a significant increase in cAMP was produced. This suggests that the effect of phenylephrine on protein kinase activity is most likely due to an undetectable increase in cAMP, capable of inducing a small but measurable increase in the protein kinase activity ratio. We found the protein kinase activity ratio to be a better indicator of cAMP elevation than measurement of cAMP itself, as discussed previously (9). All effects of phenylephrine on glycogen phosphorylase activation in heart muscle can be explained by the increase in cAMP-dependent protein kinase activity.

Hutson *et al.* (34) have recently reported that *alpha* adrenergic stimulation of isolated liver cells produced an activation of phosphorylase with no detectable increase in cAMP-dependent protein kinase activity (35). This activation of phosphorylase most likely occurs by a mechanism different from that reported here, which depends on prior activation of cAMP-dependent protein kinases.

Cyclic GMP elevation occurs in a number of tissues in response to *alpha* adrenergic stimulation (36–38). We have found that phenylephrine produces a significant increase in cGMP levels in the perfused rat heart and that this increase is blocked by phentolamine but not by propranolol. Whether this increase in cGMP might be responsible for the activation of cAMP-dependent protein kinase seen with phenylephrine concentrations which give no detectable increases in cAMP is not known. This possibility seems unlikely, in view of the high cGMP concentration necessary to produce activation of cAMP-dependent protein kinases *in vitro* (39).

A stimulatory action of adrenergic amines on glucose utilization in a number of tissues has been reported (30, 31). Williamson (40) has demonstrated that adrenergic stimulation of perfused rat heart will increase glucose uptake. In this paper we provide evidence that this stimulatory effect is due to the *alpha* component of epinephrine, since it was blocked by phentolamine but not by propranolol.

It is concluded that (a) phenylephrine will activate heart cAMP-dependent protein kinase and that this activation is due primarily, although perhaps not entirely, to *beta* adrenergic activity of the drug; (b) *alpha* adrenergic stimulation partially suppresses *beta* adrenergic activation of protein kinase; (c) activation of heart glycogen phosphorylase by phenylephrine is due to prior activation of cAMP-dependent protein kinase; (d) *alpha* stimulation increases myocardial cGMP levels; and (e) *alpha* adrenergic stimulation increases glucose transport in the perfused rat heart.

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