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EFFECT OF GLUCAGON ON RAT HEART ADENYL CYCLASE

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Abstract—Washed particulate preparations of adenyl cyclase from rat heart were used to catalyze the formation of cyclic 3′,5′-AMP from ATP in the presence of Mg^{++} and caffeine. Similar preparations from other tissues have been previously used to study the effects of a variety of hormones on adenyl cyclase. In the present study, the effect of glucagon on heart adenyl cyclase was compared to that of epinephrine in the same experiments. Glucagon was about 1·5 times more effective than L-epinephrine on a molar basis in stimulating cyclic 3′,5′-AMP formation. The effects of glucagon and epinephrine were not additive indicating a single adenyl cyclase activity in the preparations. The effect of L-epinephrine but not of glucagon could be blocked by beta-adrenergic blocking agents. These observations indicated that these hormones increased heart adenyl cyclase activity by different mechanisms. Cyclic 3′,5′-nucleotide phosphodiesterase was effectively blocked with caffeine in the incubation mixtures. In the absence of caffeine, moderate amounts of added cyclic 3′,5′-AMP were hydrolyzed by this enzyme, but this was similar in the absence and presence of glucagon.

THE INOTROPIC and chronotropic actions of glucagon were first described by Farah and Tuttle using dog heart–lung and isolated atrial preparations from several species.[1] Reports have since appeared describing the inotropic and chronotropic effects of glucagon *in vivo*, perfused hearts and isolated papillary muscle and atrial preparations.[2–4] Whereas the action of epinephrine was blocked by beta-adrenergic blocking agents, dichloroisoproterenol (DCI) or propanolol, the effect of glucagon was unaltered in the studies of Glick *et al.*[4] Murad *et al.*[5, 6] proposed that the action of catecholamines in heart is mediated via the increased formation of cyclic 3′,5′-AMP. The relative potencies of various catecholamines and the effect of adrenergic blocking agents and methyl xanthines, inhibitors of cyclic 3′,5′-nucleotide phosphodiesterase,[7] on the accumulation of cyclic 3′,5′-AMP with heart adenyl cyclase preparations support this hypothesis. Furthermore, Robison *et al.*[8] observed that in perfused rat hearts, after administration of epinephrine, an increase in cyclic 3′,5′-AMP concentration preceded the increased contractility. The reader is referred to a recent review by Sutherland *et al.*,[9] which describes in more detail the evidence relating cyclic 3′,5′-AMP to the inotropic effect of catecholamines.

The stimulatory effect of glucagon on liver adenyl cyclase is well known.[10] The effect of glucagon on the formation of cyclic 3′,5′-AMP by particulate fractions from liver was unaffected by the adrenergic blocking agents, DCI and ergotamine.[11] Glucagon also increased the formation of cyclic 3′,5′-AMP from ATP by particulate preparations

from rat adipose tissue, and this effect was unaltered by DCI.* The present study was undertaken to examine the effects of glucagon on heart adenyl cyclase with and without the presence of adrenergic blocking agents. The effects of glucagon were compared with those of epinephrine in the same experiments. The studies presented indicate that glucagon and epinephrine are capable of stimulating heart adenyl cyclase by different methods, since the effect of epinephrine but not of glucagon can be prevented with adrenergic blocking agents.

METHODS AND MATERIALS

Crystalline glucagon (Lot No. 258-234B-167-1) was provided by Eli Lilly & Co. through the courtesy of Dr. O. K. Behrens. Concentrated stock solutions of glucagon were stored in 0·01 M Tris or 0·1 M glycyl glycine (pH 9·0) at −20° and diluted in bovine serum albumin (40 μg/ml) prior to use. L-Epinephrine bitartrate was purchased from Nutritional Biochemicals Co.; 1-(3′,4′-dichlorophenyl)-2-isopropylaminoethanol hydrochloride (DCI) was purchased from Aldrich Chemical Co. Phenoxybenzamine hydrochloride (dibenzyline) was purchased from Smith, Kline & French Laboratories. D-L-Propanolol hydrochloride was obtained from Ayerst Co. Other materials were obtained as previously described.[6]

Washed particulate preparations of rat heart adenyl cyclase were prepared in 0·25 M sucrose and used to catalyze the formation of cyclic 3′,5′-AMP, as previously described.[6] In brief, particulate preparations were incubated in duplicate in disposable culture tubes in the presence of 0·04 M Tris buffer (pH 7·4), 0·016 M caffeine, $6{\cdot}6 \times 10^{-3}$ M $MgSO_4$, 4×10^{-3} M ATP, with or without hormones and blocking agents as indicated, at 30° for 10 min with gentle shaking. The quantity of particles in each milliliter of incubation mixture was derived from 35 mg of ventricular muscle. The mixtures were then heated at 100° for 3 min and centrifuged. The supernatant fractions were assayed for cyclic 3′,5′-AMP by the phosphorylase activation assay as modified by Butcher *et al.*,[12] except that preparations of inactive phosphorylase from pig liver rather than dog liver were used.

RESULTS

The time course of the accumulation of cyclic 3′,5′-AMP in incubation mixtures in the absence of hormone and with supramaximal amounts of L-epinephrine (10^{-4} M) and glucagon (10 μg/ml) is illustrated in Fig. 1. At 5, 10 or 15 min, approx. twice as much cyclic 3′,5′-AMP had accumulated in the presence of either L-epinephrine or glucagon as in the control incubations. The increased amount of cyclic 3′,5′-AMP in incubations with glucagon was still present when heated extracts of incubation mixtures were partially purified by using Dowex-2 column chromatography as previously described.[6] This indicated that the glucagon effect was not due to a nonspecific activation of the cyclic 3′,5′-AMP assay. By using a 10-min incubation period, the log–dose response curves for L-epinephrine and glucagon were compared in three different experiments and are summarized in Fig. 2. The cyclic 3′,5′-AMP formed in control incubations in these experiments was 226 ± 14 picomoles/ml. Glucagon was about 1·5 times more effective than epinephrine on a molar basis in increasing cyclic 3′,5′-AMP formation in these experiments. However, both epinephrine and glucagon

* M. Vaughan and F. Murad, manuscript in preparation.

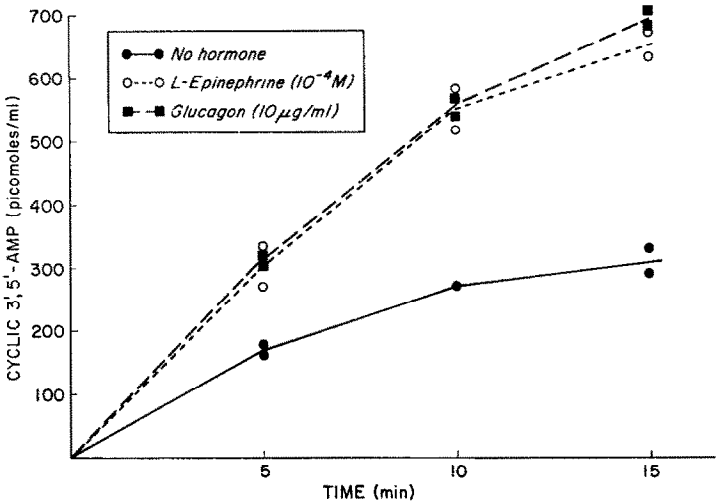

FIG. 1. Effect of L-epinephrine and glucagon on rat heart adenyl cyclase. Washed particulate preparations of rat heart adenyl cyclase were incubated in duplicate in the presence of 0·04 M Tris buffer (pH 7·4), $6{\cdot}6 \times 10^{-3}$ M $MgSO_4$, 4×10^{-3} M ATP, 0·016 M caffeine, with and without L-epinephrine (10^{-4} M) or glucagon (10 μg/ml; $2{\cdot}86 \times 10^{-6}$ M) at 30° for the times indicated. heated at 100° for 3 min and centrifuged. The supernatant fractions were assayed for cyclic 3′ 5′-AMP as described.

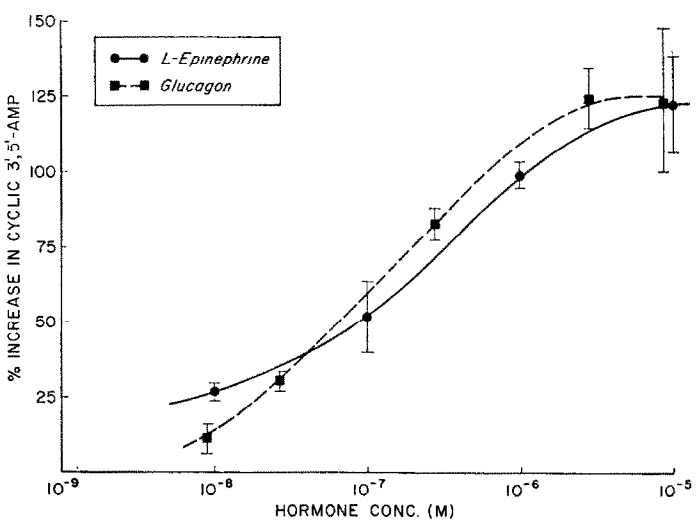

FIG. 2. Log-dose response curves for L-epinephrine and glucagon. Particulate preparations of rat heart adenyl cyclase were incubated in duplicate under the conditions obtained in Fig. 1 for 10 min without and with L-epinephrine or glucagon at the concentrations indicated. The curves represent the results from 3 different experiments in which L-epinephrine and glucagon were simultaneously compared. The bars represent the standard error of the mean. The mean of the controls in these experiments was 226 ± 14 picomoles/ml.

produced the same maximal effect and were equipotent in this respect. A maximal effect of epinephrine (10^{-4} M) was not additive to the maximal effect of glucagon (10 μg/ml) as illustrated in Table 1. This would support the hypothesis of a single adenyl cyclase activity in rat heart.

TABLE 1. EFFECT OF L-EPINEPHRINE AND GLUCAGON ON RAT HEART ADENYL CYCLASE*

Addition	Cyclic 3′,5′-AMP (picomoles/ml)
None	264
L-Epinephrine (10^{-4}M)	540
Glucagon (10 μg/ml)	534
L-Epinephrine + glucagon	560

* Particulate preparations of rat heart adenyl cyclase were incubated in duplicate as described in the legend for Fig. 2 with and without L-epinephrine (10^{-4}M) and glucagon (10 μg/ml).

The effect of glucagon on the accumulation of cyclic 3′,5′-AMP could result from either (1) increased formation of the nucleotide or (2) decreased destruction by either cyclic 3′,5′-nucleotide phosphodiesterase or some unknown pathway. To test the second possibility, washed rat heart particles were incubated under the conditions described above, but in the absence of ATP to eliminate the role of cyclic 3′,5′-AMP formation, and in the presence of added cyclic 3′,5′-AMP (720 picomoles/ml). At zero, 10, 20 and 30 min, the incubations were heated at 100° for 3 min and the remaining cyclic 3′,5′-AMP was assayed as described. Under these conditions, no cyclic 3′,5′-AMP was hydrolyzed at 10, 20 or 30 min in the presence or absence of glucagon (10 μg/ml). This indicated that the phosphodiesterase present was effectively blocked by caffeine (0·016 M) and that other pathways for cyclic 3′,5′-AMP destruction either were absent or played a minor role under these conditions. Since the phosphodiesterase activity in some preparations of heart adenyl cyclase was not completely blocked by caffeine at this concentration, the effect of glucagon in the absence of caffeine was also examined. Without caffeine, significant hydrolysis of cyclic 3′,5′-AMP occurred at 10, 20 and 30 min, as illustrated in Fig. 3. The degree of hydrolysis at 20 and 30 min was similar in the presence or absence of glucagon (10 μg/ml). At 10 min, 79 and 70 per cent of the added cyclic 3′,5′-AMP was hydrolyzed in the absence and presence of glucagon respectively. This small difference was considered insignificant and could not account for the doubling in cyclic 3′,5′-AMP levels due to glucagon in standard incubation mixtures. These experiments indicated that glucagon had no appreciable effect on cyclic 3′,5′-AMP destruction under these conditions. However, the possibility that glucagon altered the destruction of cyclic 3′,5′-AMP by another pathway which required ATP cannot be excluded by these studies.

As shown in Table 2, neither DCI, propanolol, nor dibenzyline alone at 2×10^{-5} M had an appreciable effect on cyclic 3′,5′-AMP formation. L-Epinephrine at 10^{-6} M, a concentration which produced about a half-maximal increase in cyclase activity, was totally blocked by either DCI or propanolol (2×10^{-5} M), but was unaffected by

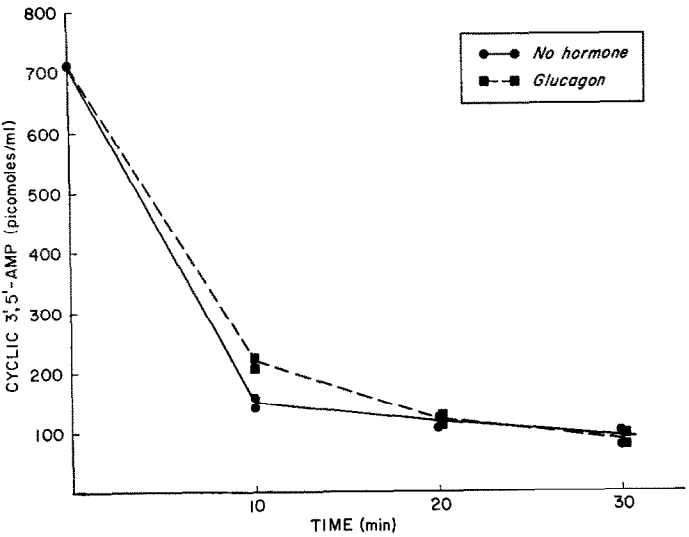

FIG. 3. Effect of glucagon on cyclic nucleotide phosphodiesterase activity. Particulate preparations were incubated under the same conditions as in Fig. 1, except for the absence of ATP and caffeine in the incubation mixtures. Some vessels contained glucagon (10 μg/ml). All vessels contained added cyclic 3′ 5′-AMP (720 picomoles/ml).

TABLE 2. EFFECT OF ADRENERGIC BLOCKING AGENTS ON ADENYL CYCLASE ACTIVITY*

Addition	Cyclic 3′,5′-AMP (picomoles/ml)
None	278
DCI	282
Propanolol	242
Dibenzyline	248
L-Epinephrine	402
L-Epinephrine + DCI	228
L-Epinephrine + propanolol	234
L-Epinephrine + dibenzyline	384
Glucagon	472
Glucagon + DCI	460
Glucagon + propanolol	472
Glucagon + dibenzyline	458

* Particulate preparations were incubated in duplicate as described in the legend for Fig. 2 with and without L-epinephrine (10^{-6} M) or glucagon (3 μg/ml) and with and without various adrenergic blocking agents at 2×10^{-5} M.

dibenzyline (2×10^{-5} M). Glucagon (3 μg/ml) produced about two-thirds of the maximal response in this experiment and its effect was unaltered by DCI, propanolol and dibenzyline (2×10^{-5} M). In other experiments, dibenzyline at 10^{-4} M produced some partial blockade of the epinephrine effect without influencing the action of glucagon. DCI, propanolol and dibenzyline at these concentrations had no effect in the cyclic 3′,5′-AMP assay in the presence or absence of added commercial cyclic

3′,5′-AMP. Therefore, the apparent blockade of the epinephrine effect by these agents was not due to an inhibitory effect in the assay.

DISCUSSION

The data presented are in agreement with the hypothesis that the inotropic and perhaps the chronotropic effects of glucagon in heart preparations are mediated via the increased formation of cyclic 3′,5′-AMP. Glucagon was slightly more effective than L-epinephrine in stimulating cyclic 3′,5′-AMP formation at concentrations that produced half-maximal responses. In dog heart–lung preparations, Farah and Tuttle found glucagon to be more effective than D-L-epinephrine on a molar basis.[1] The failure of beta-adrenergic blocking agents to block the effect of glucagon on adenyl cyclase is consonant with the observations of LaRaia *et al.*[3] and Glick *et al.*,[4] who found the inotropic effects of glucagon unaltered by DCI and propanolol in functioning heart preparations.

At the time this manuscript was submitted, Namm and Mayer[13] reported that glucagon increased heart cyclic 3′,5′-AMP levels and contractility. In these studies the beta-adrenergic blocking agent, pronethalol, was without effect on either response, while DCI prevented the inotropic effect of glucagon without altering the increased cyclic 3′,5′-AMP level. These studies are in contrast to the studies of Glick *et al.*[4] Namm and Mayer[13] suggested that glucagon may produce several biochemical and physiological effects in heart.

From the presented observations, a single adenyl cyclase activity appeared to be present in rat heart preparations, and the adrenergic blocking agents tested prevented the effect of epinephrine but not of glucagon. Therefore, it seems probable that epinephrine and glucagon act at different "receptor" sites. Whether there are separate allosteric binding sites for these hormones on adenyl cyclase, or adenyl cyclase is at least one step removed from the beta-adrenergic receptor, remains to be determined. Earlier findings that choline esters decrease adenyl cyclase activity in heart preparations in the presence or absence of catecholamines may be similarly interpreted.[5, 6] The inhibitory effect of choline esters was blocked by atropine but unaffected by DCI. Also, the stimulatory effect of catecholamines was blocked by DCI but unaffected by atropine.[6, 11]

In order to further evaluate the role of adenyl cyclase and cyclic 3′,5′-AMP in the mechanism of action of glucagon in heart, cyclic 3′,5′-AMP levels in functioning preparations with and without glucagon and beta-adrenergic blocking agents will have to be reexamined.

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REFERENCES

1. A. FARAH and R. TUTTLE, *J. Pharmac. exp. Ther.* **129**, 49 (1960).
2. T. J. REGAN, P. H. LEHAN, D. H. HENNEMAN, A. BEHAR and H. K. HELLEMS, *J. Lab. clin. Med.* **63**, 638 (1964).
3. P. J. LARAIA, R. J. CRAIG and W. J. REDDY, *Am. J. Cardiol.* **21**, 107 (1968).
4. G. GLICK, W. M. PARMLEY, A. S. WECHSLER and E. S. SONNENBLICK, *Circulat. Res.* **22**, 789 (1968).
5. F. MURAD, T. W. RALL and E. W. SUTHERLAND, *Fedn Proc.* **19**, 192 (1960).

6. F. MURAD, Y. M. CHI, T. W. RALL and E. W. SUTHERLAND, *J. biol. Chem.* **237**, 1233 (1962).
7. R. W. BUTCHER and E. W. SUTHERLAND, *J. biol. Chem.* **237**, 1244 (1962).
8. G. A. ROBISON, R. W. BUTCHER, I. ØYE, H. E. MORAN and E. W. SUTHERLAND, *Molec. Pharmac.* **1**, 168 (1965).
9. E. W. SUTHERLAND, A. G. ROBISON and R. W. BUTCHER, *Circulation* **37**, 279 (1968).
10. E. W. SUTHERLAND and T. W. RALL, *Pharmac. Rev.* **12**, 265 (1960).
11. F. MURAD, *Dissertation*, Western Reserve University (1965).
12. R. W. BUTCHER, R. J. HO, H. C. MENG and E. W. SUTHERLAND, *J. biol. Chem.* **240**, 4515 (1965).
13. D. H. NAMM and S. E. MAYER, *Pharmacologist* **10**, 145 (1968).