REGULATION OF MYOCARDIAL CYCLIC AMP BY ISOPROTERENOL, GLUCAGON AND ACETYLCHOLINE*

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Summary

A method for preparing rat and calf heart slices which retain their responsiveness to various hormones is described. It is shown that isoproterenol and glucagon independently and maximally increased myocardial cyclic AMP, up to about 6-fold, that the stimulatory action of these two hormones was not additive, and that propranolol abolished the action of isoproterenol but not that of glucagon. These data suggest that isoproterenol and glucagon interact with functionally distinguishable receptors on myocardial adenyl cyclase.

Acetylcholine antagonized the action of isoproterenol, of glucagon, or of both, greatly reducing the elevation of myocardial cyclic AMP. Acetylcholine also lowered the basal level of cyclic AMP slightly.

The actions of many hormones have been related to their ability to regulate the intracellular cyclic AMP levels in their target organs. In heart, the positive inotropic action of the catecholamines and glucagon has been suggested to result from activation of the myocardial adenyl cyclase (1-6). The present communication describes a method for preparing rat and calf heart slices retaining their responsiveness to hormones (isoproterenol, glucagon and acetylcholine), provides evidence for the multiple nature of the hormone receptors in myocardial adenyl cyclase, and shows regulation in opposite directions of myocardial cyclic AMP by physiologically antagonistic adrenergic and cholinergic agents.

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Materials and Methods

Six Sprague-Dawley rats, each weighing 200-250 g, were anesthetized with ether and the hearts were quickly excised after decapitation. Each rat had been injected subcutaneously with 0.5 mg reserpine (in 0.2 ml water) 24 hours prior to the experiment. The ventricles were sliced (0.5 mm thick) with a Stadie-Riggs tissue slicer (Arthur H. Thomas, Co.), and the slices thus obtained were further diced into pieces of 0.5 mm, using a McIlwain tissue chopper (Brinkmann Instruments). In experiments in which the effect of hormones on myocardial cyclic AMP levels were to be studied, employing the isotope prelabeling method, the slices were suspended in 30 ml of Krebs-Ringer bicarbonate medium and incubated for 40 minutes at 37° with shaking, under a gas phase of 5% CO_2-95% O_2 (v/v), in the presence of 300 μCi adenine-2-^{3}H (14 Ci/mmole, Schwarz/Mann). After the incubation, the medium containing excess radioactive adenine was removed by centrifugation. The slices were resuspended in 30 ml of the fresh medium (containing no radioactive adenine) and the latter was quickly removed by centrifugation. This washing procedure was repeated five times. The slices were finally transferred to 30 ml of the fresh medium containing 3 mM theophylline and stirred constantly to yield a near homogeneous suspension. One-ml aliquots of the suspension (containing about 15-20 mg tissue protein) were transferred by means of an automatic syringe to the incubation tubes containing 10 μl of hormones whose actions were to be studied, and the incubation was allowed to proceed for 3 min. The reaction was terminated by the addition of 0.1 ml of 50% trichloracetic acid, and the heart tissue was homogenized directly in the incubation tubes fitted with ground glass pestles. The precipitate was removed by centrifugation and the supernatant fluid was extracted three times with two volumes of ether. The radioactive cyclic AMP in the supernatant was purified first by the $BaSO_4$ method (7) following the addition of 0.5 μmole cyclic AMP as carrier. After the $BaSO_4$ precipitate was removed by centrifugation, cyclic AMP in the supernatant was chromatographed on

AG 50W-X8 columns (0.5 x 8.0 cm), the columns being eluted with water. Cyclic AMP was collected in the 6th through 8th ml fraction, the pooled fractions were treated once more with $BaSO_4$, and the precipitate was removed by centrifugation. One-ml aliquots of the supernatant were counted for radioactive cyclic AMP. In typical experiments, about 15% of the added radioactivity (as adenine-2-^3H) was incorporated into heart slices of which up to 0.1% was found in the cyclic AMP fraction. This method, based upon prelabeling the myocardial ATP pool by incubating the heart slices with radioactive adenine and measuring the formation of radioactive cyclic AMP derived therefrom, was essentially the same as originally reported for adipose cells (8,9). In experiments in which the absolute level of cyclic AMP was assayed with the protein kinase method (10), the step of prelabeling heart slices with radioactive adenine was usually omitted.

Fresh calf hearts were obtained from a local slaughter house and transported back to the laboratory in ice. Preparation and incubation of atrial and ventricular slices of calf were the same as described for rat heart slices. Protein was measured by the method of Lowry et al. (11).

Results and Discussion

Both isoproterenol and glucagon increased, in a dose-dependent manner, the accumulation of radioactive cyclic AMP in rat ventricular slices (Table 1, Experiment 1). At 0.1 μM, the lowest concentration tested, the hormones increased myocardial cyclic AMP by more than 2-fold. At a supramaximal concentration (10 μM), the stimulation by the combination of these two hormones was not any greater than when the individual hormones were present alone (Experiment 2), suggesting that they may interact independently with functionally distinguishable receptors on myocardial adenyl cyclase sharing a common catalytic subunit of the enzyme. The degree of elevation of cyclic AMP evoked by isoproterenol and glucagon was comparable whether this process was studied with the prelabeling method (which measures radioactive cyclic AMP) or by the protein kinase method (which

TABLE I. Increase in rat ventricular cyclic AMP stimulated by isoproterenol and glucagon.

Experiment	Hormone	Cyclic AMP* found by Prelabeling method	Protein kinase method
		cpm/mg protein	pmoles/mg protein
1	None	175 ± 25	
	Isoproterenol, 0.1 μM	384 ± 16	
	1 μM	850 ± 16	
	10 μM	960 ± 29	
	None	180 ± 7	
	Glucagon, 0.1 μM	467 ± 14	
	1 μM	714 ± 21	
	10 μM	783 ± 43	
2	None	293 ± 11	
	Isoproterenol, 10 μM	1865 ± 141	
	Glucagon, 10 μM	1284 ± 71	
	Isoproterenol + glucagon	1835 ± 208	
3**	None	363 ± 16	1.42 ± 0.42
	Isoproterenol, 10 μM	2691 ± 189	8.18 ± 0.56
	Glucagon, 10 μM	2389 ± 72	8.03 ± 1.46

*Each value represents mean ± S.E. of triplicate incubations.

**Two-tenth ml of the trichloracetic acid extract (total 1 ml) of the tissue from the prelabeling experiment was used for the measurement of cyclic AMP level by the protein kinase method.

measures total cyclic AMP) (Experiment 3). These data are taken as an indication that the ATP newly synthesized from the radioactive adenine was in equilibrium with the existing ATP pool(s) used for the production of cyclic AMP.

As shown earlier with adipose cells (12) employing a similar prelabeling

TABLE II. Differential effects of propranolol on rat ventricular cyclic AMP levels increased by isoproterenol (10 μM) and glucagon (10 μM).

	Cyclic AMP found*		
Propranolol	Control	Isoproterenol	Glucagon
μg/ml	cpm/mg protein		
0	363 ± 49	2691 ± 189	2389 ± 72
1	318 ± 17	1721 ± 81	2401 ± 187
10	351 ± 15	664 ± 20	2580 ± 88

*Each value represents mean ± S.E. of triplicate incubations.

technique, propranolol, a beta adrenergic blocker, blocked the elevation in myocardial cyclic AMP due to isoproterenol, while the elevation due to glucagon was not affected by this agent (Table 2). These data suggest that these two hormones may interact on separate and independent receptors on heart plasma membrane, supporting the hypothesis of multiple hormone receptors in heart advanced by others using particulate (2,3) or solubilized (13) adenyl cyclase preparations. Multiple hormone receptors have also been proposed by us (10,12) and by others (14,15) for rat adipocyte adenyl cyclase.

George et al. (6) have reported that in perfused rat hearts acetylcholine caused an increase in the basal level of cyclic GMP accompanied by a slight decrease or no change in the basal level of cyclic AMP. In the present study with incubated rat ventricular slices, acetylcholine was found to lower effectively the increased cyclic AMP levels evoked by isoproterenol, glucagon, or both (Table 3). A small decrease in the basal level of cyclic AMP in the presence of acetylcholine was observed in rat ventricular slices (Table 3) as well as in calf ventricular and atrial slices (Table 4).

TABLE III. Diminution by acetylcholine of the increase in rat ventricular cyclic AMP due to glucagon, isoproterenol, or both.

Hormones	Cyclic AMP level*		Acetylcholine effect
	Control	Acetylcholine (10 μM)	
	pmoles/mg protein		
None	2.69 $\pm$ 0.42	1.91 $\pm$ 0.46	$p < 0.1$
Isoproterenol (10 μM)	11.07 $\pm$ 0.78	7.54 $\pm$ 0.96	$p < 0.01$
Glucagon (10 μM)	5.20 $\pm$ 0.15	3.99 $\pm$ 0.10	$p < 0.001$
Glucagon + isoproterenol	11.74 $\pm$ 0.87	9.54 $\pm$ 1.36	$p < 0.05$

*Each value represents mean $\pm$ S.E. of triplicate incubations.

TABLE IV. Diminution by acetylcholine of the increase in cyclic AMP level due to isoproterenol in calf ventricular and atrial slices.

Isoproterenol	Acetylcholine	Cyclic AMP	
		Ventricle	Atrium
μM	μM	pmoles/mg protein	
0	0	27.5	16.0
0	10	19.6	10.1
0.3	0	39.2	31.6
0.3	10	20.8	15.7
10	0	49.5	43.7
10	10	21.4	19.6

The absolute levels of cyclic AMP were much higher in the calf than in the rat heart slices. This difference in absolute levels may have been due to

the time elapsed between death of the animals and commencement of incubation (rat, 1 hour; calf, 3 hours). Acetylcholine also effectively lowered the elevated myocardial cyclic AMP evoked by isoproterenol in both calf ventricle and atrium. These data obtained with rat and calf heart slices may account, in part, for the negative chronotropic and inotropic actions of acetylcholine in heart.

Laraia and Reddy (16) have studied the effects of some hormones on heart slices using a prelabeling method. Under their experimental conditions, the stimulation by norepinephrine was low and their heart preparations did not respond to glucagon or acetylcholine. The present paper reports for the first time a preparation of heart slices which retains responsiveness to catecholamine, glucagon and acetylcholine. This tissue preparation may be used conveniently for studying certain of the metabolic and functional properties of heart. Investigations concerning the regulation of myocardial cyclic AMP and cyclic GMP by various cardiac agents are now in progress.

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