Cyclic-AMP in the Perfused Failing Guinea Pig Heart[1] (35662)

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The role of 3′,5′-cyclic adenosine monophosphate (cyclic-AMP) in mediating certain metabolic effects of catecholamines on heart muscle (1) has been well established; and it has been suggested that cyclic-AMP mediates their positive inotropic effect as well (2). We have shown that activity of adenyl cyclase, regulating synthesis of cyclic-AMP, may be diminished in homogenates from failing myocardium (3). Accordingly, in the present study we measured myocardial cyclic-AMP concentration in 26 isolated perfused hearts obtained from guinea pigs with experimentally induced congestive heart failure and from 31 controls to determine whether cyclic-AMP accumulation in response to epinephrine is reduced in the intact failing heart.

Methods. Animal procedures. Frank congestive heart failure, manifested by cardiomegaly, pulmonary edema, and ascites was produced in male guinea pigs (weighing between 500 and 600 g) by supravalvular aortic constriction with a Teflon clip as previously described (3, 4).

Isolated perfused empty beating heart preparations were prepared conventionally (5) with the use of hearts from normal animals, sham operated controls, and animals with aortic constriction 1 week after operation and perfused at a constant pressure of 120 cm of H_2O with Krebs–Henseleit solution (KH) (6) oxygenated with 95% O_2, 5% CO_2 at 37°. After a control perfusion period of 20 min with KH alone, the hearts were stimulated by means of 15-sec infusions of serial dilutions of *l*-epinephrine bitartrate in KH administered with the use of a constant infusion pump. Controls were conducted with comparable infusions of KH alone. Epinephrine concentration in the coronary circulation was calculated from epinephrine infusion rate and the measured retrograde aortic flow. Left ventricular samples were obtained with precooled Wollenberger clamps and frozen in isopentane cooled with liquid nitrogen.

The effects of graded doses of epinephrine on cardiac performance of hearts from control animals and those with aortic constriction were studied in separate experiments in which left ventricular pressure (LVP), dP/dt, and heart rate (HR) were measured in isovolumic perfused hearts (7). Isovolumic hearts were not used for cyclic-AMP assays because of interference by the intraventricular balloons in the chemical studies.

Myocardial cyclic-AMP was measured by two independent methods: a modification of Brooker's enzymatic radioisotopic displacement method (8); and a recently described procedure (9) based on cyclic-AMP activation of skeletal muscle protein kinase. For both procedures samples were weighed, pulverized, extracted with 5 ml/g of 0.6 *N* perchloric acid (0°) containing 500 cpm/ml of ^{3}H-cyclic-AMP (sp act = 2.3 Ci/mmole, added for calculation of recovery), homogenized at 0° in a Sorvall macro-Omnimixer in four 15-sec bursts at a speed setting of 10, and centrifuged for 10 min at 12,000 rpm in a Sorval SS-34 rotor. The supernatant fraction was removed, neutralized with potassium hydroxide to pH 7.5, allowed to stand for at least 1 hr at 0°, and treated with barium hydroxide and zinc sulfate as described by Krishna *et al.* (10).

For assay by Brooker and co-workers' (8) method the barium–zinc supernatant fraction

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was chromatographed on Dowex-50 (8 ml of resin, 1-cm i.d. column) at room temperature. Cyclic-AMP was eluted with H_2O and applied to a Dowex-1 column (2 ml of resin, 5 × 80-mm column). Cyclic-AMP was eluted with HCl (pH 2.28), evaporated to dryness at 40°, resuspended in 100 μl of 0.15 *M* Tris (pH 8.0), and assayed in duplicate as described by Brooker *et al.* (8). Recovery, calculated on the basis of ^{3}H-cyclic-AMP initially added, averaged 50%.

For cyclic-AMP analysis by the skeletal muscle protein kinase activation method, the initial acid-soluble tissue extract was neutralized with potassium hydroxide and applied to a Dowex-50 column (8 ml of resin, 1-cm i.d.) to remove excess salt. Cyclic-AMP was eluted with water, and assayed in duplicate exactly as described by Wastila *et al.* (9).

Protein determinations on all fractions and standards were performed using the Lowry *et al.* (11) procedure modified for assay of acid-insoluble precipitates.

Results. Infusion of epinephrine produced 90% of the maximum obtainable increase in cardiac performance in isovolumic hearts within 15 sec at each dose. LVP, *dP/dt,* and HR increased in failing and control isovolumic preparations and HR increased consistently in control and experimental empty beating hearts following all epinephrine doses used in this study. Based on these results, cyclic-AMP was routinely determined in samples from empty beating hearts obtained exactly 15 sec after onset of epinephrine infusion.

Cyclic-AMP content was similar in control and failing hearts under basal conditions, averaging 7.4 and 8.1 pmoles/mg of protein, respectively. Since no differences were found between hearts from normal animals compared with sham operated controls, data from the two groups were combined. Values obtained in perfused hearts corresponded closely to those obtained in biopsies from well-ventilated, open-chest animals (Table I). In isolated perfused hearts from control animals and those with aortic constriction, changes in cyclic-AMP content in response to epinephrine infusion resulting in a final concentration in coronary perfusate ranging from 0.001 to 2.5 μg/ml were also similar (Fig. 1). The range was 9.3 ± 0.92 (mean ± SE) to 18.1 ± 1.62 and 8.7 ± 1.07 to 16.9 ± 1.48 pmoles/mg in control and failing hearts, respectively. As shown in Fig. 1, hearts from animals with aortic constriction accumulated slightly less cyclic-AMP at several dose levels. However, in no case were these differences significant or of major magnitude. The dose–response curve obtained with failing hearts did not differ significantly from that of controls when compared by nonlinear analysis of variance.

TABLE I. Myocardial Cyclic-AMP Concentration.[a]

Source of LV sample	Control	Aortic constriction
Open-chest animal	6.80 ± 0.28 (4)	7.30 ± 0.32 (4)
Langendorff preparation	7.42 ± 0.61 (5)	8.15 ± 0.60 (6)

[a] Results expressed are picomoles of cyclic-AMP per milligram of myocardial protein (mean ± SE) based on duplicate determinations in samples from the number of hearts indicated in parentheses.

These results were obtained using a modification of Brooker's cyclic-AMP assay method (8) and were corroborated by additional experiments in which myocardial cyclic-AMP concentration was assayed in the same samples by an independent method employing a

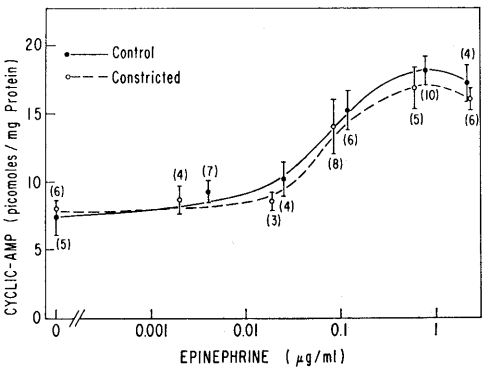

FIG. 1. Myocardial cyclic-AMP following epinephrine administration. Cyclic-AMP was assayed by the enzymatic radioisotopic displacement method as described in the text. Results are means ±SE with the number of experiments indicated in parentheses in each case.

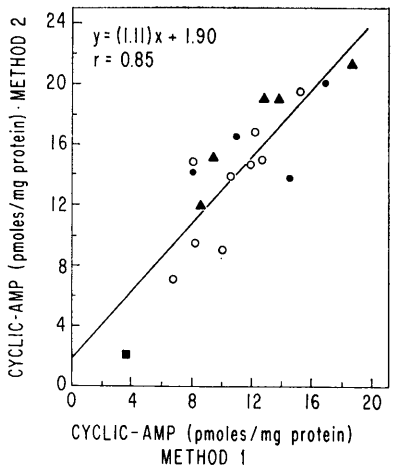

FIG. 2. Comparison of myocardial cyclic-AMP assayed by two methods. Cyclic-AMP was assayed by the enzymatic radioisotopic displacement method (shown on the abcissa), and the protein kinase activation procedure (shown on the ordinate). Results are expressed as picomoles per milligram of protein. Samples from normal animals (○); sham operated controls (●); and animals 1 week after aortic constriction (▲) were obtained after the hearts had been perfused with solutions containing graded amounts of epinephrine as described in the text. One sample (■), incubated with phosphodiesterase for 30 min, was assayed by both methods. The equation for the regression line (—) relating results obtained by these two independent assay procedures is shown.

cyclic-AMP dependent protein kinase from skeletal muscle (9). As shown in Fig. 2, results obtained with the use of both methods correlated well in representative samples from control and failing hearts. The slight apparent systematic difference between the two assay methods may be associated with slightly different treatments required to prepare the samples for analysis in each case.

Discussion. Although the mechanisms mediating catecholamine effects on myocardial function are still incompletely understood, it has been established that some depend on augmentation of myocardial cyclic-AMP synthesis (1). Since we have demonstrated previously that activity of "adenyl cyclase" is depressed in whole homogenates and particulate fractions from failing guinea pig hearts (3), the possibility was raised that diminished cyclic-AMP synthesis in response to catecholamine stimulation might occur in the failing heart. However, results obtained in the present study demonstrate that cyclic-AMP concentration *in situ* is similar in normal and failing guinea pig heart muscle; that normal and failing hearts perfused by the Langendorff technique contain similar amounts of cyclic-AMP under basal conditions; and that normal and failing perfused hearts exposed to comparable doses of epinephrine accumulate cyclic-AMP to a similar extent.

It has been suggested that several cellular systems manifesting adenyl cyclase activity exist in liver (12) and perhaps in heart muscle (13). Accordingly, it is possible that variations in specific pools of intracellular cyclic-AMP, not detectable by changes in total myocardial cyclic-AMP content, may influence cardiac performance. Although a significant reduction of adenyl cyclase activity was previously observed in homogenates from failing guinea pig hearts (3), in the present study no significant differences were noted in cyclic-AMP content of the failing heart exposed to exogenous epinephrine compared to controls (Fig. 1). In addition, total myocardial cyclic-AMP content was maintained at normal levels in the absence of exogenous catecholamines in failing hearts during 20-min perfusion periods, despite the fact that myocardial catecholamine stores of such preparations are markedly reduced (14). Thus, it appears that the altered adenyl cyclase activity observed in whole homogenates or tissue fractions is not associated with corresponding changes in cyclic-AMP synthesis in the intact heart. Although this dissociation may reflect altered lability of adenyl cyclase and loss of activity during tissue fractionation, it may indicate that total adenyl cyclase activity measured *in vitro,* is not a definitive index of the rate of cyclic-AMP synthesis in the intact heart. Ample precedent exists in related systems demonstrating dissociation between extent of activation of an enzyme and the rate of the reaction it catalyzes *in vivo,* such as the dissociation between the extent of phosphorylase activation and the rate of glycogenolysis induced by variation in substrate and adenylates (15). Tissue constituents, such as

adenosine (16) are capable of modulating the rate of synthesis of cyclic-AMP. Thus, it is not necessarily surprising that the rate of cyclic-AMP synthesis in the intact failing heart stimulated by epinephrine is not diminished in parallel with the previously observed decrease in myocardial adenyl cyclase activity in particulate fractions.

Summary. Myocardial cyclic-AMP was measured by Brooker's enzymatic isotopic displacement method and an independent method employing skeletal muscle protein kinase in hearts from guinea pigs with congestive heart failure induced by thoracic aortic constriction, sham operated controls, and normal animals under basal conditions and 15 sec after graded doses of epinephrine. Cyclic-AMP content was 7.4 ± 0.6 (mean ± SE) and 8.2 ± 0.6 pmoles/mg of protein in failing and normal hearts, respectively, under basal conditions. With maximum epinephrine stimulation, values increased to 18.1 ± 0.9 and 16.9 ± 1.5, respectively. There were no significant differences in cyclic-AMP content in failing, compared to normal, hearts at any epinephrine dose. Results indicate that there is no impairment in the overall capacity of the intact, failing heart to synthesize cyclic-AMP in response to exogenous catecholamines.

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