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OSCILLATIONS OF cAMP WITH THE CARDIAC CYCLE

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SUMMARY. Oscillations of cAMP with the cardiac cycle were demonstrated in the rat heart using a stimulator-triggered rapid freeze-clamp to decrease the temperature of the heart from 37° C to -80° C in 5 msec (20,000°/sec) at a predetermined phase of the cardiac cycle. The nucleotide, cAMP, oscillated 60% with the cardiac cycle during normal working conditions, the higher cAMP value occurring during systole.

INTRODUCTION. Although it is well known by inference that the formation of crossbridges and thus myosin ATPase activity fluctuates with the cardiac cycle, there have been few studies on other biochemical oscillations because of limitation in techniques for making such analyses. The oscillation of free intracellular calcium and high energy phosphates have only been recently demonstrated (1,2). Rapid changes in the fluorescence of aequorin in the presence of free calcium made the former possible and gated NMR the latter. Oscillations in cAMP have been shown to occur in the frog heart at a cool temperature where the heart beats slowly (3,4). Because of limitation in techniques these studies were not extended to the more rapidly beating mammalian heart, and because of experimental conditions results were never applied to mammalian physiology.

MATERIALS AND METHODS. The isolated working rat heart model as used for these studies has been recently described (5), as well as the stimulator-triggered rapid freeze clamp (6). The afterload chamber was set at 100 mm Hg and the preload chamber at 15 mm Hg.

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Coronary flow collected from the right ventricular outflow tract was 15 ml/min/g wet wt for normal conditions and 20 ml/min/g wet wt when isoproterenol (10^{-8} M) was added to the media. Media entered the left atrium and was ejected out the aorta. The heart was paced at 260 beats/min for normal conditions and 320 beats/min when isoproterenol was present. Specific details for freezing the tissue at a preselected phase of the cardiac cycle have been recently described (6), including measurement of temperature in the heart during "smashing". The center of the heart drops from 37° to -80° C in 5 msec with the techniques used here.

Frozen tissue was collected from the anvils following "smashing" in a flask containing liquid nitrogen. Tissue was pulverized under liquid nitrogen. An approximate 100 mg sample was removed, weighed, dried and weighed again for assessing wet to dry weight of tissue. Acid (3 ml 10% PCA) was pulverized separately and then added to the pulverized tissue. Pulverization under liquid nitrogen continued. The frozen mixture was transferred to a Sorvall tube and weighed, then to a mortar and brought to 0°C while pulverizing. The tissue and acid mixture were then centrifuged to remove $KClO_4$, and the supernatant stored at -80° C.

The cyclic nucleotide, cAMP, was analyzed by a radioimmunoassay as described earlier (7).

ANALYSIS AND RESULTS. Through the use of a newly developed stimulator-triggered rapid freeze-clamp which drops the temperature of the heart from 37° C to -80° C in 5 msec, it has become possible to analyze biochemical changes in the working heart under normal physiological conditions at a predetermined phase of the cardiac cycle. The relationship of the "smasher" to the heart and the perfusion apparatus is shown in Figure 1.

Results demonstrate for the first time that cAMP oscillates with the cardiac cycle in the rat heart (Figure 2). At the end of diastole prior to membrane activation, cAMP values in the perfused heart working under normal physiological conditions were 6.8 ± 1.1 (SD) nmoles/g dry wt. Following freeze clamping at max dF/dt, approximately 60 msec after stimulation, cAMP values were 10.5 ± 0.3 (SD) nmoles/g dry wt. Since beta adrenergic agonists have been shown to increase cAMP values, isoproterenol (10^{-8}M) was added to the perfusate; free calcium in the perfusate was increased from 2.5 mM to 3.5 mM to allow for maximal crossbridge formation. Under these conditions cAMP values increased to 12.5 ± 2.2 (SD) nmoles/

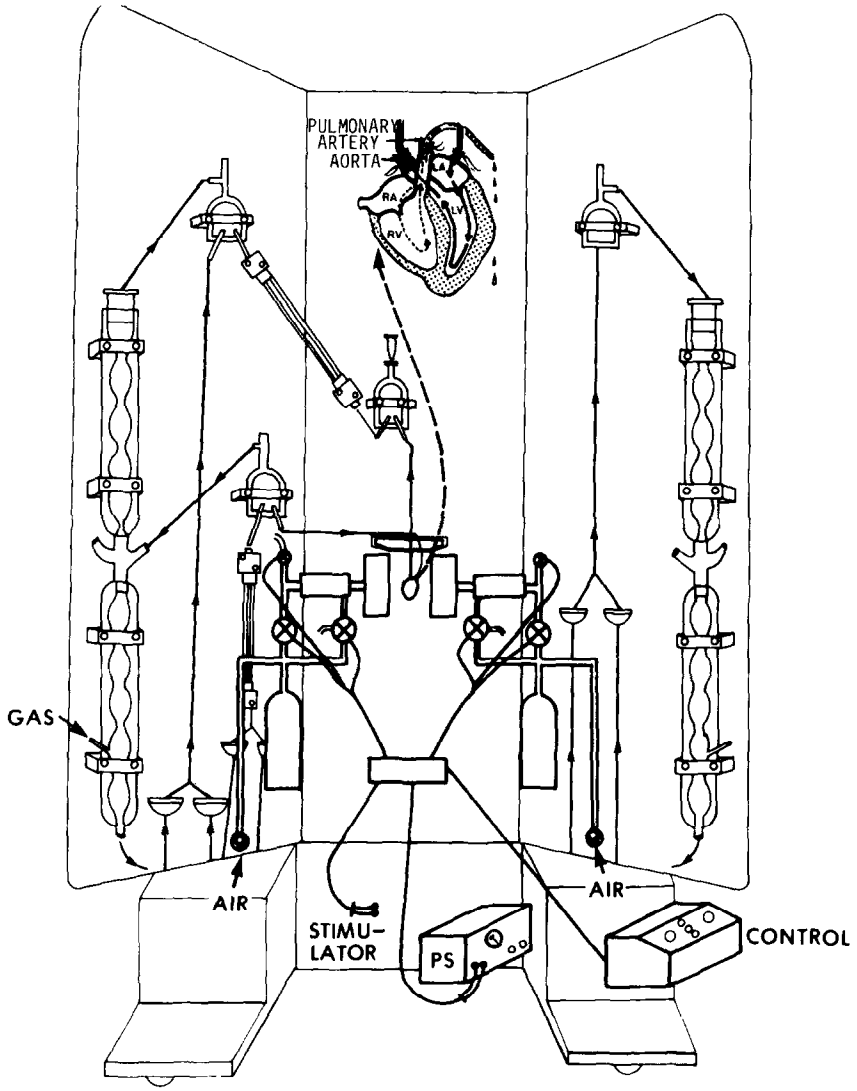

Figure 1. Stimulator-triggered freeze-clamp bolted on the face of a working heart perfusion apparatus. The perfusion apparatus is a modification of that described by Neely (14). Arrows indicate direction of perfusate flow. Duplicate perfusion set-up are shown on either panel. A complete description of the perfusion apparatus and freeze-clamp have been described in detail earlier (5,6). Media enters the left atrium and is pumped out the aorta as shown in the upper panel. The pulmonary artery is cannulated for collecting coronary flow.

g dry wt during diastole and then rose to 22.2 ± 4.2 (SD) nmoles/ g dry wt during peak force. Left ventricular pressure tracings are shown in Frames A, C, and E for the respective cAMP values shown in Frames B, D, and F (Figure 2). Arrows indicate the point of "smashing".

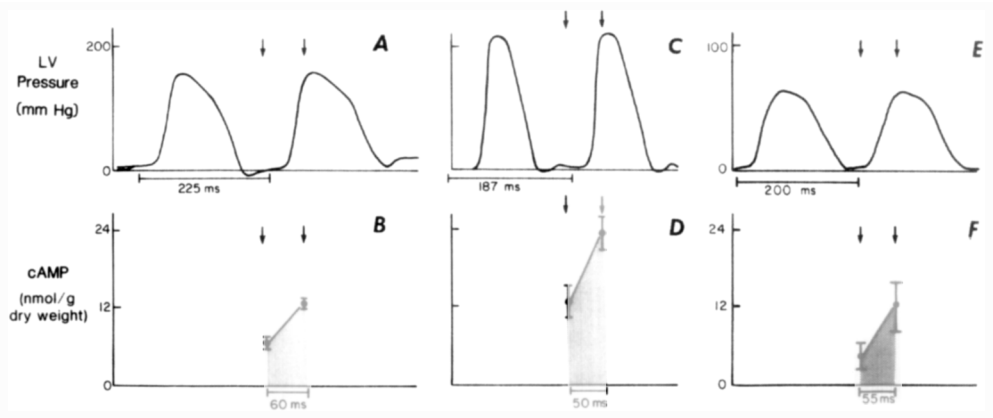

Figure 2. Pressure tracings and corresponding myocardial cAMP values are shown. Rat hearts were perfused for 20 min, then freeze-clamped at point in the cardiac cycle as indicated by the arrows, i.e., at the end of diastole 10 msec prior to stimulation and at peak force. Tissue was pulverized under liquid nitrogen, an acid extract prepared and cAMP determined by a radioimmunoassay as described earlier (7). In A and B 14 rat hearts were perfused with 2.5 mM free calcium and in C and D 14 rat hearts were perfused with 3.5 mM free calcium and 10^{-8} M isoproterenol. In E and F 14 rat hearts were perfused with 0.8 mM free calcium. The perfusion media was a modified Krebs Henseleit media as described by Neely et al (14). Fluctuations and basal concentrations of cAMP were statistically different ($p < .01$) according to the unpaired t-test. Separate experiments were carried out to assure cAMP was not modified during purification following thawing, i.e., aliquots of cAMP were added to portions of the homogenate and then subsequently quantitated

Since the concentration of free calcium in the perfusate influences contractility and relaxation rates, we were interested in determining whether a reduction of free calcium in the perfusate from 2.5 mM to 0.8 mM would also influence cAMP fluctuations. At the lower calcium concentration cAMP levels during diastole were 4.3 $\pm$ 2.3 (SD) nmoles/g dry wt and rose to 11.5 $\pm$ 3.4 (SD) nmoles/g dry wt during max dF/dt (Figure 2E and F). These results demonstrate that cAMP concentrations vary during the resting state as well as with the cardiac cycle depending on the level of the contractile state. Because we have recently shown that the substrate pyuvate obviates cyclical changes in high energy phosphates with the cardiac cycle (8), pyruvate was substituted for glucose in the perfusate; pyruvate, however, did not alter oscillations in cAMP.

There is a steep steady state differential between extracellular and intracellular calcium concentrations in the myocardium; during diastole the extracellular calcium concentrations are approximately 10,000 times higher than free calcium in the cell. Following an electrical stimulus to the myocardium accompanied by membrane depolarization there is a rapid influx of calcium and a sarcoplasmic reticulum release (9), thus increasing intracellular free calcium from approximately 10^{-7} to 10^{-5} M, depending on biochemical and physiological conditions (10). Following the influx, calmodulin binds calcium (4 moles/mole protein) and is subsequently activated. Active calmodulin stimulates receptor proteins. It has been proposed for the brain that active calmodulin in the brain stimulates adenylate cyclase thereby catalyzing the conversion of ATP into the intracellular messenger cAMP (11). After cAMP fulfills its message bearing activity it is degraded by phosphodiesterase which in turn is activated by calmodulin. Both the breakdown enzyme, phosphodiesterase and the synthesizing enzyme, adenylate cyclase, are activated by calmodulin (11). The studies described here were carried out to determine if a mechanism similar to that described above for brain may occur in the heart with membrane depolarization.

The influx of calcium with membrane depolarization appears to increase intracellular cAMP levels, perhaps through calmodulin activation (11). An elevation in cAMP stimulates protein phosphorylation. Phosphorylation of sarcoplasmic reticulum via cAMP increases the SR rate of calcium sequestration, thereby influencing relaxation rates (9); cAMP-dependent phosphorylation of troponin I decreases the calcium sensitivity of thin filaments (12), thereby limiting crossbridge formation at low calcium concentrations. Thus, cAMP may partially regulate time to relaxation by augmenting the movement of calcium from the myofilaments to the SR, via protein phosphorylation, followed by a subsequent extracellular calcium transport (13).

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