CYCLIC CHANGES IN LEVELS OF CYCLIC AMP AND CYCLIC GMP IN FROG MYOCARDIUM DURING THE CARDIAC CYCLE[1]

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SUMMARY. Cyclic AMP and cyclic GMP were measured in the ventricles of frog hearts that had been instantly frozen in situ by an automatic device at six predetermined points of the electrocardiogram. Cyclic AMP levels rose and cyclic GMP levels fell during the first three quarters of the R - T interval, which is a period corresponding to the latency phase of contraction and early phase of mechanical systole. At the time of the crest of the T wave, just after the ventricles had begun to relax, both nucleotides had returned to the preceding diastolic levels.

In a previous communication (1) we reported on oscillations of the levels of a number of metabolites in the frog heart that occurred in the course of the contraction-relaxation cycle. Significant changes took place during initiation of systole; they could summarily be expressed as a decrease in the phosphate potential, which is the ratio of the concentration of ATP to the product of the concentrations of ADP and orthophosphate, and an increase in the mass action ratio of the phosphofruktokinase reaction. These observations made us wonder, whether the levels of other myocardial

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constituents, known or suspected to have a rapid turnover, might also fluctuate in a systematic manner during the cardiac cycle. The present report deals with fluctuations of this type that concern the two cyclic ribonucleotides, adenosine 3',5'-monophosphate (cyclic AMP) and guanosine 3',5'-monophosphate (cyclic GMP).

METHODS

Rana temporaria were used in the Moscow laboratory in the month of February. They were despinalized and the chest was opened. Body temperature was 10° C, the average heart rate was 20/min. The animals were kept in this state for 3 minutes. The electrogram and mechanogram of the heart were monitored and in some cases recorded during this time. Then the hearts were practically instantly fixed with the aid of an automatically operating apparatus (2), in which a silver cylinder filled with liquid nitrogen is shot at a preset point of the electrocardiogram against the heart, compressing it to a thin frozen layer. The points selected for fixation are indicated by the vertical lines drawn across Fig. 1. A total of 142 frogs were treated in this way.

Weighed pieces of the frozen hearts consisting exclusively of ventricular muscle were finely pulverized and the powder was extracted with 20 volumes of ice-cold 5 % trichloroacetic acid. The two cyclic nucleotides in the extracts were determined in the Berlin laboratory by protein binding assays, using Gilman's (3) method for the measurement of cyclic AMP and a method (4) for cyclic GMP that utilizes a cyclic GMP-dependent protein kinase from the fat tissue of the pupa of the silk moth, Bombryx mori, and possesses the advantage, among other features, of being impervious to the presence of cyclic AMP.

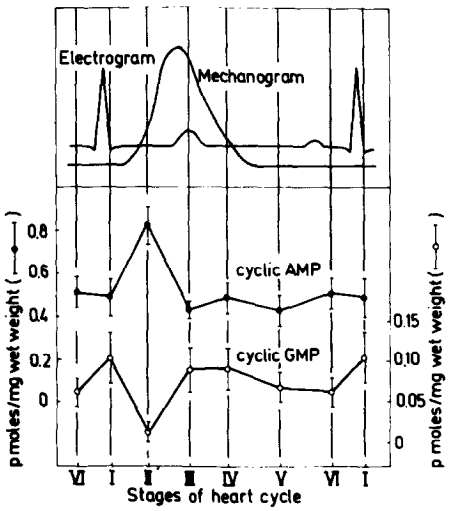

Fig. 1. Levels of cyclic AMP and cyclic GMP in the frog ventricle at six different stages of the cardiac cycle. The circles and vertical bars represent each the mean ± standard error of the results of 5 to 9 triplicate determinations. Extracts of three ventricles that had been frozen at the same stage of the electrocardiogram were pooled for the parallel determinations of the two nucleotides. The duration of the heart cycle was 3.0 sec.

RESULTS

The results of the cyclic nucleotide determinations are summarized in Fig. 1. Cyclic AMP rose during the first three quarters of the R - T interval of the electrocardiogram, which is a period coinciding with the latency phase of contraction and a major part of the rising phase of the mechanogram. The rise amounted, on the average, to 70 per cent. Simultaneously, the level of cyclic GMP fell by almost 90 per cent, from 103 to 12 picomoles, on the average, per gram of tissue. These changes were of rather short duration, lasting at most for 0.55 seconds. At the time of the crest of the T wave, marking a point (No. III in Fig. 1) when the ventricles had just started to relax, both nucleotides had returned to the preceding diastolic levels. The changes in both cyclic AMP and cyclic GMP were of statistically significant magnitude, the probability that the differences be-

tween the respective mean values at points I and II and between those at points II and III were due to chance alone being in all cases less than 2 per cent.

DISCUSSION

The data on cyclic AMP shown in Fig. 1 are in good qualitative agreement with the results of Brooker (5), who reported that the cyclic AMP level in electrically driven frog ventricle strips that were frozen in "systole" was approximately 30 per cent higher than in strips frozen in "diastole". Treatment of the strips with a near maximally effective concentration of epinephrine (10^{-5} M) produced marked rises in cyclic AMP, but the differences between the "systolic" and "diastolic" levels, far from tending to vanish, became even greater (6). The latter finding would seem to militate against the possibility that the systolic increases in cyclic AMP noted by Brooker (5) and in the present study were due to a release of endogenous epinephrine, which is the chief sympathetic neurotransmitter in the frog heart and a compound well-known for its ability to raise cyclic AMP levels in heart muscle through its ß-adrenergic stimulatory effect on adenylate cyclase (7).

Acetylcholine, the transmitter substance at the parasympathetic nerve endings, and other cholinergic agents have been found to raise in cardiac tissues the levels of cyclic GMP (8-11) and to lower those of cyclic AMP (11-13), particularly when the latter are elevated as a result of an adrenergic intervention (9, 10, 13). Conversely, the cyclic GMP-raising action of acetylcholine can be antagonized by ß-adrenergic stimulation (9). Cyclic nucleotide levels in the heart therefore probably reflect to a certain extent the balance between adrenergic and

cholinergic influences. The ventricles of the frog heart are supplied with parasympathetic as well as with sympathetic nerve fibers and contain appreciable amounts of acetylcholine for release on parasympathetic (vagal) stimulation. If we eliminate, on the strength of Brooker's (6) data on epinephrine-treated ventricle strips, a cumulation of adrenergic stimuli as a likely factor in the observed systolic changes in cyclic AMP and cyclic GMP, it is still possible to conceive of a temporary reduction in vagal tone just prior to and at the start of systole, as a cause of these short-lasting changes. However, it is difficult to deduce from available information on the action of acetylcholine and its congeners upon cardiac cyclic nucleotides, whether this action can be of sufficient rapidity and magnitude to account for the changes depicted in Fig. 1.

An explanation for the presystolic and early systolic rise in cyclic AMP that comes to mind is that this rise could have been elicited by the parallel electrical event of excitation. Electrical pulses have been shown to produce a marked accumulation of cyclic AMP in cerebral tissue (14). Application of these pulses during exposure to cyclic AMP-raising biogenic amines results in a more than additive elevation of cyclic AMP levels (14), an effect reminiscent of the potentiation of the systolic increase in cyclic AMP by epinephrine that was observed by Brooker (6) in electrically stimulated frog ventricular muscle. What effect, if any, electrical stimuli applied to excitable tissues have on cyclic GMP levels, remains to be determined.

The present findings are suggestive of a regulatory influence of cyclic nucleotides on the myocardium (15) that is exerted in a pulsatory fashion during each individual contrac-

tion cycle (16). The synchronization of positive cylic AMP and negative cyclic GMP pulses, clearly evident from Fig. 1, could then be regarded as another instance of reciprocal movements of the levels of the two cyclic nucleotides in their presumed capacity as antagonistic regulators of cellular activities (17).

Finally, the curves depicted in Fig. 1 attest to an extremely rapid turnover of cyclic AMP and cyclic GMP in frog heart muscle and point to the necessity of defining, for a correct appraisal of cyclic nucleotide levels in this muscle and possibly also in more rapidly contracting myocardium of other species, the particular phase of the cardiac cycle in which the tissue is fixed for analysis.

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