STIMULATION OF 3',5'-CYCLIC AMP FORMATION IN DOG MYOCARDIUM FOLLOWING ARREST OF BLOOD FLOW

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Received July 8, 1969

Introduction and Summary

The following changes leading to, or resulting from accelerated glycogenolysis have been observed in our laboratory in the heart of the dog in acute myocardial ischemia: Activation of glycogen phosphorylase and accumulation of intermediary and end products of glycolysis (1), activation of phosphorylase b kinase (2), and release of endogenous cardiac noradrenaline (3,4). The enzymic activations and the stimulation of glycolytic metabolism could largely be suppressed by prior administration of adrenergic ß receptor blocking agents. It was concluded that these responses were to a large extent due to an adrenergic intervention. It was postulated (4), in accordance with the current concept concerning the pathway for the action of catecholamines on muscle glycogenolysis (5), that increased formation of 3',5'-cyclic AMP by adenyl cyclase was presumably the event linking noradrenaline mobilization and phosphorylase b kinase activation in the acutely ischemic heart muscle.

The present report provides evidence in support of this hypothesis. It is shown that circulatory arrest in the dog causes an immediate rapid rise in the level of cardiac cyclic AMP which apparently precedes activation of the phosphorylase system. This rise does not take place in animals treated with the ß receptor blocker, pronethalol.

Methods

Thorax and pericardium of adult dogs were opened under pentobarbital (Nembutal, gift of Abbott Laboratories, North Chicago, Ill.) anesthesia and positive pressure breathing. A first sample of the left ventricular wall was almost instantly frozen *in situ* by the cooling clamp procedure (6) at the time designated as "0 sec". Simultaneously the ascending aorta was severed with a pair of scissors. Other ventricular muscle samples of the same heart were taken in succession by the same technique 5, 10, 15, 20, and 60 sec thereafter. Dogs which had received an intravenous injection of 7 mg per kg of pronethalol (gift of VEB Fahlberg-List, Magdeburg) 20 min prior to "0 sec" were treated in the same manner.

Adenosine 3',5'-monophosphate (cyclic AMP) was determined by the method of Breckenridge (7) with the following modifications: The frozen heart muscle pieces were homogenized in the presence of 5 volumes of 5.6 % CCl_3COOH by a 10-sec treatment with the Ultraturrax disintegrator operated at full speed; alkaline phosphatase from calf intestine (gift of Boehringer Mannheim GmbH, 6 μmoles of phosphate min^{-1} ml^{-1} of incubation mixture) was used preferentially to E. coli phosphatase and potato apyrase for destruction of unwanted adenylates; the phosphatase was not destroyed by pepsin, but was denatured by

heating the incubation mixture in boiling water for 7 min. Standard cyclic AMP solutions (Sigma Chemical Company) were processed in the same way as were the tissue extracts and aliquots of the extracts containing an internal cyclic AMP standard. Phosphorylase *a* and *a* plus *b* (E.C. 2.4.1.1) were determined according to Cori and Illingworth (8) with specifications described earlier (1). Lactate was estimated according to Hohorst *et al.* (9).

Results

As shown by curve I in panel A of fig. 1, circulatory arrest in the dog very quickly entrains a rapid rise in cardiac cyclic AMP. The level of this nucleotide in the left ventricle of 13 dogs not treated with pronethalol increased, on the average, from 0.74 nmoles/g at 0 sec to 1.90 nmoles at 5 sec following transection of the aorta, the difference between these two values being statistically highly significant ($p < 0.001$). The raised level is maintained until the 20th sec and appears to decline thereafter. Similar increases in cyclic AMP were observed in circumscribed areas of the left dog ventricle made ischemic by ligation of a branch of the left coronary artery. The adjacent non-ischemic ventricular musculature exhibited no change in its cyclic AMP content. Elevations in cardiac cyclic AMP approximating in magnitude those represented by the cyclic AMP curve I in fig. 1 were also seen, in confirmation of results obtained by numerous authors in the rat and rabbit heart (see 10), in dogs given an intravenous injection of 5 µg of adrenaline per kg.

No consistent and significant change in the level of cyclic AMP occurs during the first minute of ischemia in the

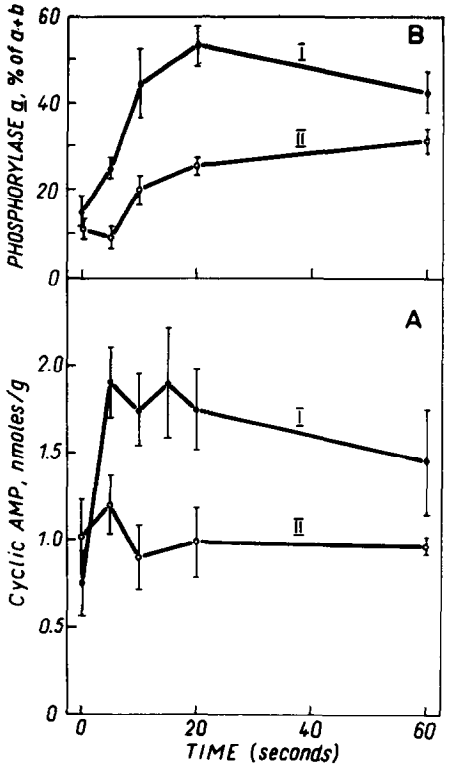

Fig. 1. Cyclic AMP and phosphorylase a levels in the left ventricle of the dog before and after the onset of myocardial ischemia. I: Dogs not treated with pronethalol; II: dogs treated with pronethalol. Number of experiments represented by the curves: AI, 13; AII, 6; BI 6; BII, 4. The points and vertical bars represent the means ± standard errors. Phosphorylase (a + b) content, measured at 30° C, averaged 14.3 μmoles phosphate g^{-1} min^{-1} in both group I and group II.

left ventricle of dogs treated with pronethalol (fig. 1, panel A, curve II). The dose of the drug given, 7 mg/kg dog, had previously been found (1) to block completely the phosphorylase activating effect of intravenously injected adrenaline and of cardiac sympathetic nerve stimulation.

Comparison of the two curves I in fig. 1 shown that the ischemia-induced formation of phosphorylase a in dog myocardium (1) is more protracted than the rise in cyclic AMP and for the most part occurs in the wake of the latter. Like the format-

ion of activated phosphorylase b kinase in ischemic dog myocardium (which is not perceptibly faster than phosphorylase a formation) (2), the process is delayed and depressed, though not eliminated, by prior administration of pronethalol (panel B, curve II; cf. ref. 2). Lactate accumulation in the myocardium of the pronethalol dogs during the first minute of ischemia was correspondingly slow (see also 1), namely, 1.9 μmoles/g as compared to 4.5 μmoles/g in the untreated dogs.

Discussion

An increase in the level of cyclic AMP in a tissue can be brought about either by acceleration of its formation from ATP through increased adenyl cyclase activity, or by slowing of its hydrolysis to 5'-AMP through a decrease in the activity of 3',5'-nucleotide phosphodiesterase, or by a combination of these two changes. The increase in cyclic AMP illustrated in fig. 1 is obtained under conditions in which liberation of endogenous cardiac noradrenaline can be demonstrated (3) and it is sensitive to the adrenergic blocking agent, pronethalol. Since catecholamines do not seem to affect phosphodiesterase and are known to increase the activity of adenyl cyclase (11), it may be concluded that the rise in cyclic AMP level seen in the ischemic dog myocardium is chiefly or entirely due to stimulation of the formation of this compound, caused by the action of liberated noradrenaline on cardiac adenyl cyclase. On this and on previous (4) evidence the rapid shift from aerobic to anaerobic energy production which takes place in dog myocardium following interruption of its blood supply can be visualized to involve, supplementary to non-humoral regulatory influences on carbohydrate enzyme activities (12), the following

sequence of events in the ischemic tissue: Liberation of endogenous catecholamine, increased formation and accumulation of cyclic AMP, activation of phosphorylase b kinase, activation of phosphorylase, acceleration of glycogenolysis and of glycolysis.

Breckenridge (7) noted that the cyclic AMP content of mouse brain was elevated after a 20-sec period of ischemia. Robison et al. (13) reported that the cyclic AMP content of the rat heart did not deviate from the aerobic value after a 3-min perfusion with oxygen-free solution. They inferred that anoxia was without effect on cyclic AMP levels in the heart. Their data, however, are not at variance with the present results, since extrapolation to 180 sec of the cyclic AMP curve in fig. 1 yields a value which likewise does not differ appreciably from the aerobic level. Whether the curve actually takes such a course and whether or not cyclic AMP fails to rise in the rat heart upon switching from aerobic to anaerobic perfusion, are points to be settled by determinations of cyclic AMP levels in the cardiac tissues at the appropriately chosen times following the onset of myocardial ischemia and hypoxia.

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