cGMP- AND cAMP-MODULATED CALCIUM BINDING BY THE MYOCARDIAL SARCOLEMMA IN CIRCULATORY HYPOXIA

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Contraction of heart muscle is initiated by membrane depolarization and consequent penetration of the contraction trigger (Ca^{++} ions) into the cytosol along the concentration gradient from the extracellular space through the sarcolemmal membrane (SM). Release of Ca^{++} ions from the cell is brought about with the aid of an Na^{+}/Ca^{++}-carrier, which works in a direction which can be varied during the relaxation – contraction cycle [5, 7], and also of sarcolemmal transport Ca-ATPase, which removes Ca^{++} from the cytosol during diastole. Data have recently been obtained to show that the intermediate stage of this last reaction is binding of Ca^{++} by protein components of SM [4]. We know that the character of this binding is modulated by cyclic nucleotide-dependent phosphorylation of Ca-binding proteins of SM [8, 11].

It is therefore very probable that cAMP- and cGMP-dependent regulation of Ca^{++} binding in the myocardial sarcolemma has a functional significance, for it determines removal of the trigger Ca^{++} from the cytosol, and thus modulates the cardiac rhythm. Meanwhile the course of this process in the presence of myocardial damage has virtually not been studied.

Accordingly, in the investigation described below, cGMP- and cAMP-modulated binding of Ca^{++} ions by SM and activity of sarcolemmal Ca-ATPase were investigated both in the intact myocardium and in the presence of a reversible lesion of heart muscle, namely circulatory hypoxia.

EXPERIMENTAL METHOD

Mongrel dogs weighing 15-18 kg were used. Circulatory hypoxia was induced by bleeding until the blood pressure was 50 mm Hg, over a period of 3 h under general anesthesia produced by 2.5% thiopental sodium solution [2]. The ischemic tissue of the left ventricle was investigated, and the same regions of myocardium from normal animals served as the control.

The sarcolemmal fraction was isolated in a sucrose density gradient by the writers' modification of the method in [9]. cAMP-dependent protein kinase was obtained as described previously [3]. The cAMP and cGMP were determined by means of kits of reagents from Amersham Corporation (England). Binding of Ca^{++} with SM was effected by a modified method in [10]. After 0.2 mg of protein had been kept for 10 min in the incubation medium in the presence or absence of 10^{-6} M cAMP, 0.2 mg protein/ml of protein kinase, and 10^{-8}-10^{-6} M cGMP, the reaction was started by addition of 20 μM Ca^{++}, containing 0.05 μCi of ^{45}Ca. Aliquots were filtered through "Millipore" filters (HA, 0.45 μ) and the quantity of bound Ca^{++} was determined as the difference between the radioactivity of the initial reaction mixture and the radioactivity of the filtrate. Activity of Ca-activated sarcolemmal ATPase was measured as the quantity of inorganic phosphorus (P_i) in the medium [10].

EXPERIMENTAL RESULTS

It will be clear from Table 1 that no significant changes were found in the basal (non-stimulated) level of Ca^{++} binding after incubation for 5 min in preparations of intact and

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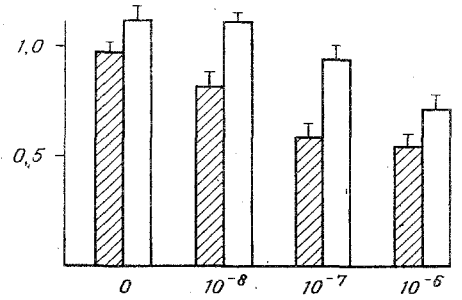

Fig. 1. Binding of Ca^{++} by myocardial sarcolemma in the presence of different concentrations of cGMP. Abscissa, cGMP concentration in incubation medium (in M); ordinate, Ca^{++} binding (in mmoles ^{45}Ca/mg protein). Shaded columns denote ^{45}Ca binding in 30 sec; unshaded columns – binding of ^{45}Ca in 5 min.

TABLE 1. Binding of Ca^{++} by Myocardial SM under Normal Conditions and in Circulatory Hypoxia

Regions of myocardium	^{45}Ca binding, nmoles/mg protein					
	basal level		in presence of cAMP		in presence of cAMP and protein kinase	
	30 sec	5 min	30 sec	5 min	30 sec	5 min
Control	0,98±0,04	1,13±0,05	1,38±0,07	1,82±0,09	1,58±0,06	2,02±0,10
Affected	0,97±0,05	1,07±0,02	1,29±0,03*	1,29±0,06*	1,52±0,06	1,94±0,10

Legend. Mean results of 5-7 determinations. *P < 0.05 compared with control.

TABLE 2. cAMP and cGMP Concentrations and Ca-ATPase Activity in Sarcolemma of Intact Myocardium and in Circulatory Hypoxia

Regions of myocardium	cAMP concentration, pmoles/g tissue	cGMP concentration, nmoles/mg protein	Ca-ATPase activity, μmoles P_i/mg protein/min
Control	635±51	5,66±0,62	0,44±0,04
Affected	547±48	7,03±0,70	0,45±0,07

Legend. Mean results of 6-9 determinations.

affected SM. Addition of 1 μM of cAMP to the incubation medium stimulated Ca^{++} binding by 61% in intact membranes, but by only 21% in circulatory hypoxia. Addition of exogenous protein kinase as well as cAMP to the incubation medium equalized the levels of ^{45}Ca binding in the affected and intact myocardium (stimulation of Ca^{++} binding by 81 and 79% respectively compared with the basal level). The observed decrease of cAMP-dependent (endogenous) activation of Ca^{++} binding in circulatory hypoxia was evidently due to disturbance of the ability of the holoenzyme protein kinase to dissociate with release of the catalytic subunit and, consequently, with a reduction in phsophorylating activity, as the writers showed previously in this form of myocardial lesion [1].

Restoration of the level of Ca^{++} binding after the addition of exogenous protein kinase (exogenous phosphorylation) to the corresponding control values (intact myocardium) is evidence of the absence of phosphate modifications to molecules of the target proteins (a change in accessibility of the regions for phosphorylation for the kinase) in the sarcolemma of the affected heart compared with SM of the intact myocardium. It is evidently reduction

of the phosphorylating activity of cAMP-dependent protein kinases which is the decisive factor in the development of disturbance of phosphorylation of target proteins in the sarcolemma, for no significant change takes place in the concentration of cAMP, a coenzyme of the protein kinase reaction, in the cardiomyocyte cytosol in this form of myocardial ischemia (Table 2).

A study of the effect of cGMP-dependent phosphorylation as well as cAMP-dependent on the process of Ca^{++} binding by the myocardial sarcolemma is of definite interest because an increase in the cGMP concentration is observed in preparations of SM in circulatory hypoxia of the myocardium (Table 2).

It will be clear from Fig. 1 that addition of 10^{-8} M cGMP to the incubation medium caused no significant change in Ca^{++} binding. Addition of 10^{-7} M cGMP to the incubation medium after 5 min of incubation led to inhibition of Ca^{++} binding by 15.9%, whereas addition of 10^{-6} M cGMP inhibited it by 36.3%. The increase in the cGMP concentration which we found in the sarcolemma of the affected heart and inhibition of Ca^{++} binding in the presence of this nucleotide by membrane proteins may play an important role in the disturbance of regulation of sarcolemmal Ca-ATPase in the ischemic myocardium. It must be pointed out that Ca-ATPase activity in SM of the affected myocardium was unchanged compared with the corresponding values in the intact heart, which correlates with the unchanged basal (nonstimulated) level of Ca^{++} binding in intact and damaged SM. This suggests uncoupling of the operation of the Ca pump of these membranes and of its cyclic nucleotide-dependent regulation in this form of myocardial lesion.

In conclusion, it will be noted that the changes in Ca^{++} binding by SM discovered in the presence of cAMP and cGMP may be a decisive factor both in the regulation of the working of the sarcolemmal Ca-pump in the intact myocardium and in the development of irreversible damage to heart muscle. This last situation may be due to the fact that oversaturation of the cytosol of the affected cell with Ca^{++} ions is the critical factor in the development of profound changes in the cardiomyocyte [6], and a contributory factor to this state may be, in particular, disturbance of the regulation of activity of the Ca-pump of SM in the ischemic myocardium.

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