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EFFECT OF ELEMENTARY SULFUR ON ADENYLATE KINASE ACTIVITY AND WORKING OF THE ISOLATED RABBIT HEART

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UDC 612.17.014.46:546.22].085

KEY WORDS: heart, sulfur, adenylate kinase, vasodilatation.

Elementary sulfur is used in industry, agriculture, and medicine. Besides as an external application, intravenous injections of sulfur in peach oil also are used in medicine for pyrogenic treatment [1]. The mechanism of its action has not been explained. It has recently been shown [2] that sulfur inactivates adenylate kinase (AK), one of the most important enzymes in energy metabolism of cells. It has also been shown [3] that sulfur has a moderate uncoupling and inhibitory effect on respiration of the cardiac mitochondria, and in combination with dithiothreitol, it inhibits adenine-nucleotide translocase. The writers previously postulated [4] a role for adenylate kinase in intracellular processes of energy transport and in the energy supply for myofibrillary contractions.

The aim of this investigation was to study the possibility that sulfur may inhibit AK in living cells and the effect of sulfur itself on the work of the isolated rabbit heart and on the coronary perfusion flow. Temporary inhibition of AK activity may also be an essential measure for preventing degradation of adenine nucleotides during tissue ischemia, thereby facilitating restoration of the working capacity of the organs in the postischemic period [5].

EXPERIMENTAL METHOD

The isolated rabbit heart was perfused by Langendorff's method under isometric conditions with modified Tyrode solution (144 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 10 mM Tris-HCl, 10 mM glucose, pH 7.4), saturated with oxygen. Throughout this period the amplitude and frequency of the cardiac contractions were recorded and the velocity of the coronary flow of perfusion fluid measured. The amplitude of the cardiac contractions was measured by means of a 6MKh IC mechanotron. Activity of AK isozymes was measured as described previously [6]. Mitochondria were isolated from the rabbit heart as in [7].

EXPERIMENTAL RESULTS

The mechanism of action of sulfur and its effect on activity of individual AK isozymes still remained unknown.

Laboratory of Metabolism, Central Research Laboratory, Kaunas Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR J. J. Bredikis.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 10, pp. 377-379, October, 1990. Original article submitted December 30, 1989.

TABLE 1. Effect of Elementary Sulfur on Physiological Parameters of the Perfused Rabbit Heart

Experimental conditions	Amplitude of cardiac contractions, mm	Frequency of cardiac contractions, beats/min	Perfusion flow rate, ml/min
Control (30 min)	23,5±1,5	128±3	19,2±1,1
Ethanol 0.4% (20 min)	21,8±2,9	117±6	20,5±2,5
			$p_1<0,01$
Ethanol 0.4% + sulfur (20 min)	15,5±1,1	114±8	41,5±4,3
	$p<0,02$		$p_2<0,05$

Legend. Mean results of 6-7 experiments are shown. P_1) Compared with control, P_2) compared with ethanol. Here and in Table 2, 1 ml of a saturated solution of sulfur in absolute ethanol per 250 ml of perfusion fluid was used; time after which corresponding measurements were made indicated in parentheses.

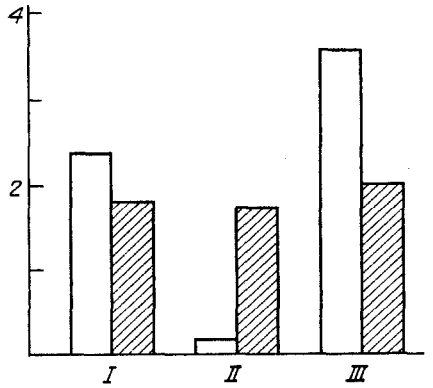

Fig. 1. Effect of elementary sulfur on activity (in μmoles ADP/min/mg × 10^{-2}) of AK_1 (unshaded columns) and AK_2 (shaded columns). I) Control, II) incubation with sulfur, III) incubation with dithiothreitol (DTT). Concentration of sulfur (dissolved in absolute ethanol) 20 μM, of DTT 1 mM. A preparation of cardiac mitochondria was used as AK_2 and the supernatant after their isolation as AK_1. Incubation time with sulfur 90 min, with sulfur + DTT 30 min.

As Fig. 1 shows, sulfur inhibits only the cytoplasmic AK isozyme (AK_1). Sulfur has no action on the activity of the mitochondrial isozyme (AK_2). The same results were obtained with fragmented (freezing and thawing) mitochondria. This shows that the absence of effect of sulfur on the mitochondrial isozyme is unconnected with the inaccessibility of this enzyme in intact mitochrondria. In the presence of dithiothreitol, inhibition by sulfur is abolished and AK_1 activity actually rises above the control level (Fig. 1). The effect of dithiothreitol shows that the effect of sulfur is connected with inhibition of the SH-groups of the enzyme. Further experiments showed (data not given) that sulfur in fact reduces the number of titratable SH-groups in AK and reacts with SH-groups of free cysteine. Data in the literature [8] indicate that only AK_1 is sensitive to SH-reagents (dithio-bis-nitrobenzoic acid etc.). It can be concluded from our own data and thos in the literature that sulfur is also an SH-reagent specific for the SH-groups of AK, since it does not act on the activity of other enzymes sensitive to SH-reagents [2].

Elementary sulfur is a lipophilic reagent, and it can therefore be suggested that it passes through biological membranes. In this connection it was interesting to examine the effect of sulfur on AK_1 activity in the intact myocardium, and also on physiological parameters of the perfused rabbit heart. Data on the effect of sulfur on the work of the perfused rabbit heart and on its coronary blood flow are given in Table 1. As these results show, if sulfur is present in the perfusion fluid the amplitude of the cardiac contractions is reduced and the flow of perfusion fluid increased. in these experiments a control for ethanol was used, since the sulfur was dissolved in it. The vasodilator effect of ethanol

TABLE 2. Effect of Elementary Suflur on AK Activity (in μmoles ADP/min/mg protein) in the Perfused Rabbit Heart

Experimental condition	Total AK activity	AK_1 activity
Control, n = 8	2,09±0,18	1,57±0,15
Perfusion with sulfur(20)	1,50±0,20	0,79±0,12
p	<0,05	<0,002

is well known, and in our experiments it also increased the flow of perfusion fluid, but in combination with sulfur, vasodilatation was increased by an additional one-third. Consequently sulfur can be regarded as a member of a new class of vasodilators. In this connection pharmacological studies of sulfur would be interesting. It must be pointed out that the action of some vasodilators known at the present time also is connected with their effect on the SH-groups of proteins [9].

Measurement of total AK activity and activity of its isozymes (TAble 2) showed that after perfusion of the heart with sulfur, total AK activity in the myocardium was reduced by 28%. This decrease was due mainly to inhibition of the cytoplasmic isozyme (AK_1), activity of which was reduced by 50%. Since the cytoplasmic isozyme of AK is involved in energy production for myofibrillar contraction, the fall of its activity may give rise to reduced contractility of the heart (Table 1). However, regulation of contraction of heart muscle is a sufficiently complex process and it is impossible to draw any unambiguous conclusions.

The experiments thus showed that sulfur inhibits the cytoplasmic AK isozyme both in vitro and in vivo (the perfused rabbit heart). The effect of sulfur is directed toward the SH-groups of the enzyme. Sulfur has a vasodilator effect on the perfused rabbit heart and depresses myocardial contractility. In combination with ethanol, sulfur may be an effective vasodilator.

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