ANTIARRHYTHMIC ACTION OF ADENOSINE AND THE CYCLIC NUCLEOTIDE LEVEL IN THE ISCHEMIC RAT MYOCARDIUM

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Two stages are distinguished in the development of ventricular arrhythmias in animals with myocardial infarction: the first corresponds to the acute period (15-30 min after coronary occlusion), the second is observed after 4-8 h and lasts 2 days. The early disturbances of rhythm, which resemble the "prehospital" arrhythmias in man, have the severest course. These arrhythmias are one of the main causes of death in the acute period myocaridal infarction. The mechanisms of development of the arrhythmias differ and have not been fully explained. One possible explanation suggests that cAMP and an associated excess of Ca^{++} intake into the cardiomyocytes during ischemia are involved. Under partial depolarization conditions due to a raised extracellular K^{+} concentration, an increase in the slow Ca^{++} inflow into the cells may lead to uncoordinated contraction of muscle fibers and to ventricular arrhythmias [6]. Meanwhile the time course of the myocardial cAMP concentration during ischemia has not been thoroughly studied. Some workers have observed a rise of the nucleotide level, coinciding in time with the development of arrhythmia [7], whereas others could not confirm these observations [1, 8].

There is no information on the cGMP concentration in the ischemic myocardium. The role of adenosine in the development of arrhythmias has not been fully explained, although in the light of data showing its anti-adrenergic and antiarrhythmic action, it has been suggested that this compound may play the role of "endogenous antiarrhythmic agent" in ischemia [2].

The aim of this investigation was to study the antiarrhythmic activity of adenosine and its possible effect on the cyclic nucleotide levels in the myocardium after coronary occlusion.

EXPERIMENTAL METHOD

Experiments were carried out on 60 noninbred male albino rats weighing 180-200 g. In the animals of the first two groups the left coronary artery was ligated at the lower border of the auricle of the left atrium under pentobarbital anesthesia (50 mg/kg, intraperitoneally), with artificial ventilation of the lungs. Immediately after ligation rats of group 1 received an intravenous infusion of adenosine (from Reanal, Hungary) in physiological saline in a dose of 2.5 mg/kg/min by means of a micropump over a period of 30 min. Animals of group 2 received a similar infusion of physiological saline at the rate of 0.07 ml/min. During the 30 min after occlusion the ECG was recorded continuously in standard lead II, and from it the number of ventricular extrasystoles, and the frequency and duration of attacks of tachycardia and ventricular fibrillation during this period were determined [11]. Intact animals of group 3 received an infusion of adenosine in a dose of 2.5 mg/kg/min for 30 min. Intact animals of group 4 were used only to determine the basal level of cAMP and cGMP.

To estimate the cyclic nucleotide concentrations pieces of myocardium were frozen in liquid nitrogen and homogenized at the 15th, 30th, and 120th minutes of the experiment. Proteins were precipitated with 6% TCA. After centrifugation at 3000 rpm the TCA was removed from the supernatant by triple extraction with water-saturated ether. The samples were lyophilized and dissolved in Tris-HCl, pH 7.5, before nucleotide assay. The cAMP and cGMP concentrations were determined by the competitive binding method using test kits from Amersham International, England.

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TABLE 1. Effect of Adenosine on Frequency and Severity of Arrhythmias (M ± m)

Group of animals	Number of animals	Number of extrasystoles	Ventricular tachycardia		Ventricular fibrillation	
			duration, sec	frequency, %	duration, sec	frequency, %
1. (Myocardial infarction + adenosine)	12	87±12*	14,3±9,8	33,3	0,7±0,05*	8,3
2. (Myocardial infarction + physiological saline)	10	136±18	15,6±4,1	100	4,6±1,2	16,7

Legend. *p < 0.05 compared with control

TABLE 2. Time Course of Cyclic Nucleotide Levels (in pmoles/mg tissue) in Rat Myocardium after Infusion of Adenosine (M ± m)

Group of animals	Test object	cAMP			cGMP			cGMP/cAMP		
		time, min								
		15	30	120	15	30	120	15	30	120
1-(Myocardial infarction + adenosine)	Zone of infarct	0,73±0,12*	0,73±0,10*	0,77±0,11*	0,026±0,0028	0,0025±0,003	0,026±0,002	0,036	0,034	0,034
	Peri-infarct zone	0,87±0,25*	0,92±0,19	0,87±0,16	0,033±0,003	0,04±0,003*	0,022±0,002*	0,045	0,043	0,025
2-(Myocardial infarction + physiological saline)	Zone of infarct	0,75±0,10*	0,83±0,16	0,62±0,20*	0,021±0,002*	0,02±0,0025*	0,018±0,0027*	0,028	0,024	0,029
	Peri-infarct zone	0,72±0,18*	0,83±0,16	1,0±0,4	0,031±0,003	0,023±0,001*	0,026±0,004*	0,047	0,026	0,025
3-(Adenosine)	—	0,91±0,2	1,0±0,36	0,97±0,07	0,036±0,0027*	0,04±0,001*	0,029±0,030	0,036	0,04	0,03
4-(Basal level)			1,0±0,2			0,029±0,004			0,029	

Legend. *p < 0.05 compared with basal level.

EXPERIMENTAL RESULTS

Disturbances of the cardiac rhythm were discovered in all rats of groups 1 and 2 (Table 1). The arrhythmias appeared 6.2 ± 0.2 min after coronary occlusion and continued until 18.3 ± 1.3 min. Injection of adenosine significantly reduced the number of extrasystoles and the duration of ventricular fibrillation, reduced the frequency of onset of ventricular tachycardia by two-thirds, and reduced the frequency of ventricular fibrillation by half.

In the myocardium of the animals of group 2 (control) the cAMP level was lowered after 15 min both in the zone of ischemia and around the periphery of the infarct (Table 2). This period coincided with the peak of development of the arrhythmias. Later the cAMP level outside the zone of the infarct returned to its initial value, but in the region of the infarct it continued to fall. The cGMP concentration after 15 min was lowered only in the region of infarct, but later it fell both in the infarct and at its periphery. The cGMP/cAMP ratio in the zone of ischemia was close to its initial value throughout the experiment, but in the peri-infarct region after 15 min it was 1.6 times higher than initially.

After infusion of adenosine into rats of group 1 a significant increase in the cGMP concentration was observed in 30 min in the zone of ischemia and at the periphery. The cAMP level in the myocardium was unchanged or even fell in the zone of the infarct after 30 and 120 min. The cGMP/cAMP ratio in the region of ischemia and, in particular, at the periphery, exceeded its initial value.

Similar results were obtained in the animals of group 3 without coronary occlusion: adenosine itself did not affect the cAMP level in the myocardium but it increased the cGMP concentration at the 15th and 30th minutes of infusion. The cGMP/cAMP ratio also increased.

Thus in experiments on rats no correlation was found between the onset of arrhythmias in acute myocardial ischemia and the cAMP concentration in the myocardium. Arrhythmias were found against the background of a reduced cAMP concentration, and the cGMP level fell simultaneously. During the first minutes of ischemia, because of changes in permeability of the sarcolemma, nucleosides and nucleotides are lost by the cardiomyocytes [9]. This may be the explanation of our observations. No correlation likewise could be found between the antiarrhythmic action of adenosine and changes in the cAMP level, although this mechanism has been suggested by some workers [4]. Meanwhile infusion of adenosine was accompanied by an

increase in the cGMP concentration in the myocardium of intact animals and of rats with coronary occlusion. The cGMP/cAMP ratio increased under these circumstances. The character of the action of adenosine resembles the effect of cholinomimetics, which also increase the cGMP concentration in the myocardium, leave the cAMP level unchanged, and raise the threshold of ventricular fibrillation [3, 10]. This response may perhaps be evidence of increased parasympathetic activity when adrenergic influences are weakened by adenosine. It has recently been shown conclusively [4] that adenosine inhibits catecholamine release from presynaptic endings and that they interact with β-adrenoreceptors. Adenosine may also be the direct cause of the increase in guanylate cyclase activity and of the intensification of cGMP synthesis. Administration of exogenous adenosine is followed by its rapid conversion into inosine, hypoxanthine, xanthine, and uric acid. During oxidation of xanthine with xanthine oxidase intensive formation of superoxide anions takes place, and these are powerful guanylate cyclase activators [5]. Meanwhile the biological importance of these changes is not yet clear, for the role of cGMP in the myocardium and other tissues has not been finally settled.

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