HEART MUSCLE ENKEPHALIN AND CYCLIC NUCLEOTIDE LEVELS DURING VENTRICULAR FIBRILLATION INDUCED BY EXPERIMENTAL MYOCARDIAL INFARCTION

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Rhythm disturbances are found in 95% of patients with acute myocardial infarction (AMI) [2]. One of the most dangerous types of arrhythmia is ventricular fibrillation [2]. However, the pathogenesis of fibrillation in AMI has not been adequately studied. It has been shown that endogenous opioid peptides may have a negative chronotropic action on the myocardium [7]. The question of the mechanism of this effect, however, remains open, and no data on the role of enkephalins in the pathogenesis of fibrillation in AMI could be found in the literature. Yet we know that enkephalins can significantly alter the metabolism of cAMP [9], whose participation in the pathogenesis of ventricular fibrillation has been proven [11]. The writers previously obtained data on an enkephalinergic rise in the myocardial level of cGMP [1], which also is a regulator of the cardiac rhythm [6].

The aim of this investigation was to study correlation between ventricular fibrillation in experimental coronary arterial occlusion and changes in the myocardial cyclic nucleotide and enkephalin levels.

EXPERIMENTAL METHOD

Experiments were carried out on 89 noninbred male rats weighing 200-250 g, in which the left anterior descending coronary artery was ligated under sodium amytal anesthesia, 3 mm below the point of its origin, by the method in [12]. On the basis of the results of continuous ECG recording for 15 min after the beginning of coronary occlusion, the animals as a whole were divided into two groups. Group 1 consisted of rats which developed ventricular fibrillation, lasting not less than 3 min, during this time interval. Rats of group 2 did not develop fibrillation while under observation for 15 min, or its total duration did not exceed 60 sec. It must be emphasized that the level of coronary arterial occlusion was the same in all the rats, but nevertheless, ventricular fibrillation developed in some animals but not in others. Other investigators also have described similar findings [8]. It can be tentatively suggested that the probability of development of fibrillation is connected with individual differences in the response of the myocardium to ischemia. In addition, in both groups of animals rhythm disturbances developed after coronary arterial occlusion in the form of atrial and ventricular extrasystoles — both single and polytopic. The development of fibrillation was found not to depend on these changes of rhythm. Intact animals anesthetized with amobarbital sodium were used as the control. Material from the animals of group 1 was obtained during fibrillation, and from animals of group 2, 15 min after coronary occlusion. Pieces of myocardial tissue were frozen in liquid nitrogen immediately after removal. Cyclic nucleotides were determined by radioimmunoassay using kits from "Amersham" (England), after preliminary ethanol extraction by the method recommended by the firm. Enkephalins were determined by radioimmunoassay using kits from the firm International Nuclear Corp. (USA) after extraction from the tissues with a 0.1 N solution of HCl in methanol. The results were subjected to statistical analysis by Student's t test and determination of the coefficient of correlation (r).

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TABLE 1. Effect of Ventricular Fibrillation on Enkephalin and Cyclic Nucleotide Concentrations in Rat Myocardium after Coronary Arterial Occlusion

Parameter	Intact animals (control)	Animals with fibrillation (group 1)		Animals without fibrillation (group 2)	
		nonischemic zone	zone of ischemia	nonischemic zone	zone of ischemia
LE, ng/g tissue	36,8±5,0 (32)	9,7±1,6 (8)	19,9±4,4 (12)	26,3±5,5 (9)	49,5±12,1 (7)
p_1	—	<0,001	<0,05	>0,05	>0,05
p_2	—	<0,02	<0,02	—	—
ME, ng/g tissue	364±70 (16)	907±305 (8)	1312±291 (9)	765±152 (12)	819±139 (12)
p_1	—	>0,05	<0,01	<0,001	<0,01
p_2	—	>0,05	>0,05	—	—
cAMP, pmoles/g tissue	498±32 (22)	533±56 (25)	668±54 (26)	665±82 (17)	453±50 (15)
p_1	—	>0,05	<0,01	>0,05	>0,05
p_2	—	>0,05	<0,01	—	—
cGMP, pmoles/g tissue	31,8±1,9 (19)	41,6±3,8 (25)	22,8±1,3 (22)	21,0±1,2 (15)	21,9±1,9 (19)
p_1	—	<0,05	<0,01	<0,001	<0,001
p_2	—	<0,001	>0,05	—	—

Legend. p_1) Significance of differences compared with intact animals; p_2) the same compared with animals without fibrillation. Number of observations shown in parentheses.

EXPERIMENTAL RESULTS

The development of acute coronary ischemia was accompanied by a significant decrease in the Leu-enkephalin (LE) content in the myocardium of the rats during fibrillation (Table 1). The reduction of this parameter in the conventionally "intact" myocardium was twice that observed in the zone of ischemia. The reason for this change in the LE content of the animals with fibrillation has not yet been explained. It can be tentatively suggested that it was the result of more intensive degradation of LE in the "intact" myocardium. In the animals of group 2 (without fibrillation) no significant changes were found in the LE level compared with the control. The data showing a fall in the LE level in the rats of group 1 showed definite negative correlation with the total duration of fibrillation: for the ischemic zone $r = -0.51$, for the nonischemic zone $r = -0.56$. On the basis of these data it can be postulated that changes in the LE concentration play a definite role in the pathogenesis of the rhythm disturbances in AMI.

Taking into consideration the data obtained previously on the ability of an LE analog to raise the cGMP level in the myocardium of rats with AMI [1], it was decided to look for possible correlation between the fall in the LE concentration observed and changes in the cGMP level.

Acute myocardial ischemia, complicated by fibrillation, gave rise to opposite changes in the cGMP concentration in different zones of the myocardium. For instance, in the zone with disturbance of the circulation a fall in the cGMP level was observed compared with that in intact animals by 28%, but in the region with its circulation intact, the level was raised by 30%. The decrease in the cGMP concentration in the zone of infarction during fibrillation and in the myocardium of rats without fibrillation could be the result of inhibition of guanyl cyclase, for activity of phosphodiesterase, which hydrolyzes cGMP, is unchanged in AMI [10]. The increase in the cGMP concentration in the nonischemic region of the heart in the animals of group 1, however, is evidently due to a simultaneous increase of tone of the parasympathetic nervous system [5]. Negative correlation was found between the cGMP and LE levels in the region outside the infarct ($r = -0.54$). Possibly LE, mobilized from peptidergic terminal in the myocardium [4], could be involved in the activation of cGMP synthesis in intact cardiomyocytes in this case. The decrease in the total content of this peptide in the heart muscle, noted above, could be a result of active degradation of free LE, released from the depots.

On the basis of the results no unequivocal solution could be obtained to the problem of the pathogenetic or, conversely, the compensatory character of the changes described. However, data in the literature on the antiarrhythmic action of the stable LE analog in experimental myocardial ischemia [3] suggest that a fall of the level of this peptide, connected with its release from the depots and intensification of its metabolism, is evidently protective and adaptive in character. This action of LE may be based on its inhibitory effect on catecholamine release from sympathetic terminals in the myocardium [4].

The existence of this close correlation between the LE and cGMP concentrations in the "intact" myocardium, correlation between the cGMP level and the duration of fibrillation

(r = +0.59), and also the information that the heart rate is reduced under the influence of cGMP analogs [6] and the cGMP level is raised in response to administration of a stable LE derivative [1], indirectly confirm our hypothesis.

The concentration of Met-enkephalin (ME) in the myocardium of rats with acute ischemia was appreciably increased (Table 1). The increase was significant in the zone of ischemia and outside it. No significant differences in the ME concentration were found in the animals of groups 1 and 2.

An important role in the mechanism of elevation of the ME level in heart muscle during AMI may be played by potentiation of activity of aminopeptidases and enkephalinases under the influence of endogenous catecholamines [4], release of which from synaptic terminals is intensified during AMI [10].

The cAMP concentration was significantly increased in the zone of ischemia in animals with fibrillation compared with its level in intact rats and animals with myocardial ischemia but without fibrillation, in agreement with data in the literature [11]. No significant correlation was found between the cAMP and enkephalin concentrations, nor between the ME level and the duration of fibrillation.

The onset of fibrillation in animals with AMI is thus accompanied by interdependent changes in the LE and cGMP levels in the myocardium, aimed predominantly at compensation of the disturbances of heart muscle excitability arising under those conditions. Elevation of the ME level in the heart tissue induced by myocardial ischemia evidently does not play a decisive role in the onset of fibrillation.

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