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DISTRIBUTION OF A NEUROSPECIFIC CARDIOACTIVE PROTEIN–HORMONAL COMPLEX IN RATS WITH EXPERIMENTAL MYOCARDIAL ISCHEMIA

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Over a long period we have collected abundant factual evidence that cardioactive protein–hormonal complexes which we have identified in the magnocellular nuclei of the hypothalamus in man and various animals [2, 4, 6] are biochemical systems which are responsible for the chemical regulation of metabolism and of the functions of various visceral organs and, in particular, cardiovascular activity [8]. On dissociation of these complexes, the high-molecular-weight component has been shown to consist of new neurospecific glycoproteins, and the low-molecular-weight compounds noncovalently bound with them are cardiotrophic neurohormones, discovered previously in the same brain region and conventionally known as K, S, and G [3].

With the aid of highly specific antisera obtained to these protein–hormonal complexes and by methods of immunoelectrophoresis [[1] and radioimmunoassay (RIA), their specificity for nerve tissue has been proved, their subcellular localization studied, and their concentrations and distributions in the rat have been determined [6]. The use of RIA has opened up fundamentally new prospects for the study of the varied functions of neurospecific complexes involved in the hormonal regulation of cardiovascular activity by the CNS under normal and pathological conditions.

The aim of this investigation was to study brain levels and regional distribution of one of these complexes, namely protein-neurohormone K (PNK) in a model of experimental myocardial ischemia (MI) in rats.

EXPERIMENTAL METHOD

Experiments were carried out on 70 noninbred male albino rats weighing 160-200 g. Experimental MI was induced by ligation of the descending branch of the left coronary artery. Maximal development of MI on the 4th day was judged by recording the ECG on a 6NEK-3 apparatus (needle electrodes, unrestrained rats). The ECG of the animals was recorded in three standard leads, before and 5 min after occlusion of the coronary artery and before sacrifice of the animals. A few minutes more after occlusion of the left coronary artery the rats exhibited marked ischemic changes: a considerable rise of the ST segment and enlargement of the T wave on the ECG in standard leads. Later, three days after occlusion of the coronary artery, signs of established MI were observed on the ECG: as a rule a QS complex or a deep Q wave would be seen.

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TABLE 1. PNK Content in Various Tissues ($M \pm m$, $n = 8$)

Tissue, organ	PNK level, ng/g wet weight of tissue	
	intact rats	rats with MI
Brain regions		
cerebral cortex (occipital)	855,0±8	7,40±0,06*
hypothalamus	1075,0±10	15,20±0,02*
medulla	236,0±6	20,00±0,15*
Cerebellum	1270,0±13	19,20±0,07*
Heart	0,86±0,12	22,50±0,86*
Lungs	0,42±0,25	19,20±0,91*
Kidneys	0,97±0,08	21,00±0,32*
Liver	0,50±0,14	30,60±0,68*
Spleen	0,39±0,14	75,00±3,23*
Pancreas	0	40,00±0,09*
Skeletal muscle	0,43±0,09	40,80±1,87*

Legend. *$p < 0.05$ compared with control.

The animals were decapitated 4 days after ligation of the coronary artery and various regions of the brain (cerebral cortex, hypothalamus, cerebellum, medulla) and visceral organs (heart, lungs, liver, kidneys, spleen, pancreas, skeletal muscle) were taken. The tissues were homogenized with water in a ratio of 1:2 (W/V) and centrifuged at 5000 rpm. Proteins were precipitated from the supernatant with ammonium sulfate between 70 and 100% of saturation. The precipitates were dissolved in Veronal-Medinal buffer (pH 8.6) and dialyzed against water for 48 h. Freeze-dried powders of the tissue extracts and blood serum were used as PNK antigen. Antiserum to PNK isolated from the hypothalamus [4] was obtained by immunizing rabbits with a mixture of this complex with Freund's complete adjuvant, using a microtechnique developed previously [1].

RIA was carried out with antiserum reacting strictly specifically with PNK. The method was based on demonstration of the PNK-antibody complex with the aid of chloramine T [19] against ^{125}I-labeled PNK. The iodinated PNK was separated by gel-filtration through Sephadex G-25 and eluted with 0.05 M phosphate buffer, containing 1% bovine serum albumin. The experiments were carried out by Bolton's method [7] with our own modifications [5]. Radioactivity of the PNK preparations was measured on an SL4221 scintillation counter (France) with calculation of a value characterizing the relationship between radioactivity and quantity of bound PNK. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The water-soluble antigenic complex PNK from brain and visceral organs was isolated by the method described above. The yield of tissue extracts, fractionated with ammonium sulfate and freeze-dried, varied from 0.1 to 0.3% of the wet weight.

Comparison of the antigenic complexes isolated from the brain and viscera of intact animals and of rats with permanent occlusion of the descending branch of the left artery revealed no difference between them. During crossed immunoelectrophoresis, rabbit antiserum to PNK from bovine hypothalamus likewise reacted with water-soluble antigenic complexes from the brain of intact animals and of rats with MI. The precipitation test, however, was negative when tissue extracts from the viscera were used as antigens. With this fact in mind, a more sensitive method of detecting PNK was used subsequently, namely RIA with ^{125}I-labeled antigen.

On the 4th day after ligation of the coronary artery there was a sharp fall in the PNK content in the various brain regions of the animals, and it was particularly marked in the cerebral cortex (about 115 times lower than in intact animals). There was a smaller decrease in the PNK content in the hypothalamus, cerebellum, and medulla — by 11-70 times (Table 1).

MI not only greatly accelerated the release of PNK from the brain, but also led to a considerable rise of its blood level from 13 ± 0.85 to 620 ± 3.9 ng/ml. The highest level of PNK for the visceral organs, about 200 times higher than initially, was found in the spleen. A marked increase in the PNK content also was found in the skeletal muscles, pancreas, liver, heart, and kidneys (Table 1). The fact that PNK was significantly increased by the 4th day of MI in these organs requires careful analysis. In this respect the study of the change in this parameter over a period of time may yield evidence of the pathogenic role of PNK and the part it really plays in metabolic processes in these organs in this particular pathology.

It can be tentatively suggested that the redistribution of the PNK content (a decrease in the brain and a sharp increase in various organs) observed in MI is evidently aimed at increasing the peripheral tissue (including myocardial) blood flow. Since in the modern view the essence of coronary insufficiency is mismatching between the oxygen supply and demand of the myocardium, and taking the preliminary data into account, it can be postulated that PNK, by greatly improving the blood supply to the heart, may reduce this mismatching. There is no doubt that quantitative determination of the PNK concentration in the blood serum and in various organs is not sufficiently informative for determining the effect of PNK in MI. However, considering the abnormally large increase in the PNK content in the heart, it can be assumed that this complex also is involved in scar formation in the zone of necrosis.

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