GLYCERINATED FIBERS OF RAT CARDIOMYOCYTES AS A MODEL FOR PRECLINICAL TRIALS OF ANTIANGINAL AGENTS

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UDC 615.224.076.9

KEY WORDS: glycerinated fibers; contractile proteins; antianginal agents.

The damaging action of ischemia in the myocardium leads to profound dystrophic changes at the myocyte organelle level [5, 11]. these changes also involve the myocardial contractile protein system, leading not only to irreversible loss of contractility of the myofilaments [1], but also to deformation and partial disappearance of actin and myosin filaments [4]. The writers showed previously [2] that treatment of animals with experimental myocardial ischemia (EMI) with various antianginal agents has a positive action on contractility of the contractile apparatus of the ischemic myocardium. The complexity of the search for and evaluation of antianginal agents has to be taken into account [7].

This paper describes an investigation into the possibility of using our modified method of obtaining glycerinated myocardial fibers (GMF) for the preclinical screening of antianginal agents.

EXPERIMENTAL METHOD

The following agents were tested on various experimental models: verapamil 200 μg/kg, propranolol 1 mg/kg, and pyrroxan 1 mg/kg. Contractility of the GMF was determined on 50 male rats weighing 180 ± 20 g, divided into 5 groups (10 rats in each group): 1) intact, 2) animals with EMI; 3) with EMI + verapamil; 4) with EMI with propranolol; 5) with EMI + pyrroxan. EMI was produced by ligation of the descending branch of the left coronary artery. After the creation of EMI, the rats were treated for 4 days with the above-mentioned drug. On the 5th day the animals were decapitated under other anesthesia and GMF obtained by the method in [13] from an intact part of the left ventricle. The effects were judged by the degree of contraction of GMF under the influence of ATP in a concentration of $5 \cdot 10^{-5}$ M. The coronary artery was occluded by the method in [10] on 28 conscious male rats, divided into four groups (7 in each group): 1) animals with EMI; 2) with EMI + verapamil; 3) with EMI + propranolol; 4) with EMI + pyrroxan. To identify the composition of the actomyosin complex obtained from the left ventricular myocardium, 80 male rats were used. They were divided into four groups (with 20 in each group): 1) intact; 2) rats with EMI; 3) with EMI + verapamil; 4) with EMI + pyrroxan. The experimental conditions were the same as for the previous series. Actomyosin was obtained by the method in [12]. Electrophoresis of proteins was carried out in 10% PAG and SDS by the method in [14].

EXPERIMENTAL RESULTS

The results of the experiments of GMF show that their contraction under normal conditions was 31.7 ± 1.1% of the initial length of the fibers, taken as 100%. Comparison of data for intact rats with data for rats with EMI showed that in the zone outside the infarct contractile proteins of the cardiomyocytes were damaged and the contractility of GMF was reduced to 21 ± 2.4% (Fig. 1).

Treatment of the rats with verapamil, propranolol, and pyrroxan led to a varied degree of improvement in the system of contractile proteins. Contraction of GMF obtained from the myocardium of these animals was considerably increased. These data are evidence of the positive effect of treatment on contractile proteins of the cardiomyocytes.

Laboratory of Pharmacology and Pathophysiology, Research Institute of Cardiology, Ministry of Health of the Armenian SSR, Erevan. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 4, pp. 444-446, April, 1989. Original article submitted May 10, 1988.

0007-4888/89/0004-0501$12.50

TABLE 1. Composition of Protein Subunits of Actomysin Complex in Experimental Ischemia and Treatment with Verapamil and Pyrroxan

Component of actomyosin complex	Intact animals (control group)	Group with EMI, untreated	Group with EMI, treated with verapamil	Group with EMI, treated with pyrroxan
Myosin heavy chain	35,6±0,98	41,5±1,28*	39,1±0,9*	24,0±0,98*
α-Actinin	7,6±0,17	5,5±0,52*	6,8±0,47*	3,8±0,26*
Actin	15,0±0,27	12,3±0,85**	18,0±0,87*	9,3±0,62*
Troponin T	1,7±0,16	2,2±0,7	3,2±0,52**	2,9±0,18*
Tropomyosin	7,2±1,03	5,95±0,12	5,1±0,21***	9,0±0,68
Troponin I	5,6±0,51	9,0±0,27*	5,8±0,38	4,5±0,68
Myosin light chain	5,5±0,17	4,3±0,44**	9,1±0,5*	4,7±0,56
Myosin light chain	6,8±0,8	6,0±0,7	9,8±1,1**	6,7±0,38
Troponin C	5,0±0,27	4,7±0,47	5,3±0,61	7,2±0,62*
Myosin light chain	0	0	0	7,5±0,85

Legend. *p < 0.001, **p < 0.01, ***p < 0.05.

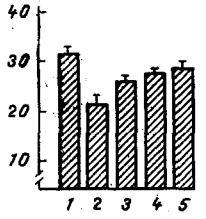

Fig. 1. Changes in contractility of glycerinated fibers during pharmacotherapy. Abscissa, index of contractility of GMF (1 – intact, 2 – with EMI, untreated, 3 – with EMI + verapamil, 4 – with EMI + propranolol, 5 – with EMI + pyrroxan); ordinate, contractility of GMF (in percent).

To confirm the antianginal action of the drugs tested in experiments on GMF, they were next studied in experiments with ligation of the coronary artery of conscious animals. Electrocardiographic data showed that two rats of the control group developed fibrillation, followed by cardiac arrest, after occlusion of the coronary artery. In all the other cases, the ECG showed evidence of focal changes in the form of a monophasic curve or the development of marked subendocardial ischemia.

In the groups receiving verapamil and propranolol, these drugs had a beneficial action. There were no deaths, but in 3 or 4 cases evidence of mild myocardial damage was obtained in the form of elevation of the ST segments and reduction of the overall voltage. The remaining animals developed signs of subendocardial ischemia.

In 2 of 7 rats receiving pyrroxan after ligation of the coronary artery signs of subendocardial ischemia were observed, whereas in the rest, reduction of the blood supply was not significant.

It can be concluded from the experimental results that the α-adrenoblocker pyrroxan was more effective than verapamil or propranolol in the treatment of acute myocardial ischemia, in agreement with the data obtained in experiments on GMF.

Since the contractility of GMF depends on the state of the contractile proteins, in order to discover the molecular mechanisms of the action of antianginal theapy, an investigation was made of the subunitary composition of the actomyosin isolated from the rats' myocardium. This enables the level of damage to the contractile apparatus of the myocardium to be identified and the degree of correction of these changes by the various cardiotropic drugs to be assessed.

Electrophoretic fractionation of the protein components of actomyosin showed that on the 5th day of EMI there was a significant increase in the relative content of myosin heavy chains, of troponin T, and of the ATPase inhibitor troponin I, and a decrease in the content of actin, tropomyosin, myosin light chains, and troponin C (Table 1).

Treatment of the animals with verapamil and pyrroxan led to dissimilar changes in the composition of actomyosin, with the exception of troponin I. Both drugs reduced its concentration in the myocardium toward the normal level.

Under the influence of verapamil a tendency was noticed for the concentrations of myosin heavy chains, α-actinin, and actin to return to normal. Under the influence of pyrroxan, however, all the above parameters were below the control levels. As regards myosin light chains, their concentration increased considerably during verapamil therapy, whereas during pyrroxan therapy their normal level was restored. It should be noted that administration of pyrroxan to animals with EMI led to the appearance of a new myosin isozyme. This fact can be regarded as transition to a new and more effective level of contraction, which is sometimes observed under the influence of extremal factors [3]. We know that during stress pyrroxan can almost completely suppress catecholamine excretion and has a marked protective action in reactions of the sympathoadrenal system, an imbalance of which is one of the leading causes of disturbances of cardiovascular function under these conditions [6].

Despite some differences in the direction of action of these drugs, they have one property in common: they restore the normal level of troponin I, indirect evidence of activation of the ATPase activity of actomyosin, and which ultimately has a beneficial effect on the properties of the contractile system as a whole.

Similar results also have been obtained with the antianginal agent propranolol [9]. The authors cited found that propranolol, with its regulating effect on the subunitary composition of actomyosin, delayed the development of necrosis in the presence of marked myocardial ischemia.

Treatment of the animals with the above-mentioned drugs thus directly or indirectly affected the rate of renewal of the myofibrillary proteins of the myocardium. The positive action of this process also was revealed on a model of GMF, contraction of which increased appreciably under the influence of treatment. Similar results were obtained in a study of the action of various cardiotropic drugs on the reaction of superprecipitation of the actomyosin complex of cardiomyocytes [8].

The effectiveness of therapeutic substances can therefore be judged indirectly on the basis of the degree of contractility of GMF of cardiomyocytes, and this method can be recommended for the preclinical screening of antianginal drugs.

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HISTAMINE RELEASING ACTION OF POLYMYXIN B AND ITS ANALOGS

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UDC 615.33:577.182.34].015.4:[616-008.953.6-008.94:577.175.824].076.9

KEY WORDS: mast cells; histamine secretion; structure and activity of polymyxins.

Among mast cell activators special importance is attached to the peptide antibiotic polymyxin B (PB) and its analogs. First, it is important to establish the connection between the histamine-releasing activity (HRA) of the polymyxins with their structure and, correspondingly, to determine sites on the antibiotic responsible for triggering secretion. Second, PB and its derivatives can be used as standard activators of the target cells of allergy and inflammation in order to discover changes in the reactivity of these cells systems in patients with allergy. Third, PB can be useful to analyze the basic principles of the secretory process. In the modern view, triggering of the secretory process in the mast cell activator molecule requires the presence of free positive charges and of lipophilic regions capable of fixation on the cell membrane [2].

In this investigation, to study relations between HRA of a mast cell activator and its hydrophobic properties and charge, we used PB and its analogs, differing with respect to these parameters.

EXPERIMENTAL METHOD

Male Wistar rats weighing 250-300 g were used. Mast cells (90-95% purity) were isolated from a cell suspension obtained from the peritoneal and pleural cavities [5]. The experimental conditions, sources of reagents, and compositions of the solutions used to incubate the cells were described previously [3]. Histamine was determined by a fluorometric method [5]. Polymyxin B_1 (PB_1) was obtained by the HPLC method on an Ultrasphere C8 column (Alltex, USA) measuring 9.5 × 250 mm in a system of 0.1 M NaCl–HCl, pH 2.0, in water-methanol 25:75. Deacylated derivatives of PB – decapeptide (DPB), nonapeptide (NPB), and heptapeptide (HPB) – were obtained by enzymic hydrolysis of the original antibiotics [1, 4, 10]. The structure of the peptides was determined by amino acid analysis after hydrolysis with 5.6 N HCl, and confirmed by identification of the 11-terminal amino acid residues in the form of their dansyl derivatives. The individuality of the compounds tested was characterized by reverse-phase HPLC as described previously [4].

EXPERIMENTAL RESULTS

The compounds studied had the following structure: R–Dab–Dab–D–Phe–Leu–Dab–Dab–Thr, where for PB_1, R = $CH_3CH_2CH(CH_3)(CH_2)_4CO$–Dab–Thr–Dab, for DPB R = Dab–Thr–Dab, for NPB R = Thr–Dab, and for HPB R = H. PB [9] is a mixture of PB_1 and PB_2; for PB_2 R = $CH_3CH(CH_3)(CH_2)_4CO$–Dab–Thr–Dab. A characteristic feature of the ploymyxins is their amphilicity, due on the one hand to the presence of positively charged diaminobutyric acid (Dab) residues, and on the other hand to fatty acid, leucine, and phenylalanine residues. By using the chosen series of compounds (PB, PB_1, DPB, NPB, and HPB) it is possible to assess the contribution of all functionally important fragments of the molecule to its biological activity.

Institute of Immunology, Ministry of Health of the USSR. All-Union Antibiotics Research Institute, Ministry of the Medical and Biological Industry of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 4, pp. 447-449, April, 1989. Original article submitted March 15, 1988.

 0007-4888/89/0004-0504$12.50