LIMITATION OF HEMODYNAMIC DISTURBANCES IN ACUTE HYPOXIA AND REOXYGENATION IN SITU BY THE SYNTHETIC PEPTIDE BIOREGULATOR DALARGIN

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UDC 616.1-009.1-02:616-008.922.1-008.64]-085.23-036.8-07

KEY WORDS: hypoxia; reperfusion; Ca-homeostasis; dalargin.

Hypoxia is the key factor in the pathogenesis of critical states of whatever etiology, but certain of the individual properties inherent in opioid peptides and dalargin, for example, the antistressor activity of dalargin [2, 4, 10], presuppose their ability to influence different stages of this process. It was accordingly decided to analyze the effect of the preventive action of the synthetic leucine-enkephalin analog (dalargin) in a model of acute respiratory hypoxia (asphyxia) in rats on the degree of restoration of myocardial contractility during reperfusion, determined by the use of an integral parameter of the circulatory system, namely cardiac output (CO), which was used as an objective functional criterion of hypoxic disturbances in the whole organism, as being responsible for the most important (circulatory) level of the oxygen transport function.

In the investigation described below, and on the basis of some phenomenologic effects of dalargin, approaches are outlined to the discovery of individual possible mechanisms whereby opioid peptides realize their stress-inhibiting properties.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats, anesthetized with pentobarbital (30 mg/kg, intraperitoneally) and artificially ventilated at the rate of 60 beats/min. Acute respiratory hypoxia was created by stopping the artificial ventilation for different time intervals: for 5, 7.5, and 10 min [9]. The period of asphyxia was followed by reoxygenation for 30 min with simultaneous recording of the time course of CO, which was determined in the rats under open chest conditions, by continuous measurement of the volume velocity of the blood flow by means of a vascular transducer (FH-015 T) of an MFY-1200 electomagnetic flowmeter ("Nihon Kohden," Japan), fixed to the ascending aorta. Curves of the heart rate (HR), stroke volume (SV), and CO were recorded on a Mingograf-800. A special series of experiments was carried out to assess the effect of dalargin on parameters of the pumping function of the isolated perfused heart (IPH) after overloading of the Ca-pump by producing an excess of Ca^{2+} in the incubation medium by replacing the original perfusion solution by hypercalcium solution [8]. The hearts were perfused by the methods in [13, 14] at a temperature of 37°C, through cannulas introduced into the left atrium and aorta, with initial values of the filling pressure and resistance of 15 and 100 cm water respectively, with standard Krebs—Henseleit solution (pH 7.3-7.4), oxygenated with carbogen (95% O_2 + 5% CO_2). The program of the experiments envisaged switching IPH from the working perfusion mode, described by Neely [14] with normal Krebs—Henseleit solution to retrograde perfusion, according to Langendorff [13], with Krebs—Henseleit solution containing a 3 times higher Ca^{2+} concentration. The final Ca^{2+} concentration in the perfusion fluid was 9 mM, with which the pH and osmolarity changes were very small and were, therefore, not compensated. The Ca^{2+} concentration throughout the period of perfusion by Langendorff's method (10 min) was monitored by means of calcium ion-selective electrodes (F-2110-Ca, Radiometer, Denmark). Dalargin, in a concentration of 108-8 V hypercalcium solution, and just as in the

A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'nol Biologii i Meditsiny, Vol. 112, No. 11, pp. 505-507, November, 1991. Original article submitted April 10, 1991.

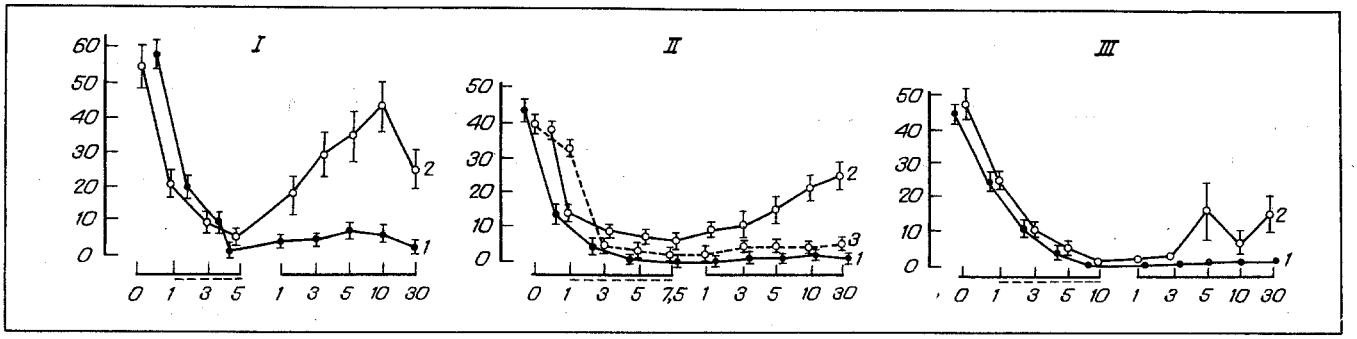

Fig. 1. Effect of preventive infusion of dalargin on CO in asphyxia-related hypoxia and subsequent reoxygenation in Wistar rats. I, II, III) Series of experiments. Abscissa — initial values of CO are projected on the zero marker; broken line — duration of asphyxia-related hypoxia (in min); continuous line — period of reoxygenation (in min). Ordinate, values of CO (in ml/min). 1) Control (placebo); 2) experiment (injection of dalarginin a dose of 100 μg/kg intravenously, 5 min before disconnecting artificial ventilation); 3) combined intravenous bolus injection of dalargin (100 μg/kg) and naloxone (500 μg/kg).

control (without addition of the opioid), retrograde perfusion of IPH was carried out for 10 min, followed by replacement of the normal Krebs—Henseleit solution on switching IPH to the original perfusion by Neely's method.

EXPERIMENTAL RESULTS

Asphyxia for 5 min led to a marked decrease in the values of CO, HR, and SV, which depended on time and, by the end of the 5-min period of hypoxia, amounted to 5, 15, and 25% respectively of the initial level before discontinuing the artificial ventilation. Subsequent resumption of respiration did not lead to any marked recovery of cardiac activity, i.e., all the parameters studied remained at the same low level as before application of artificial ventilation before the end of the period of enforced asphyxia.

In those experimental animals which received an infusion of dalargin (100 μg/kg, intravenously) 5 min before the beginning of asphyxia, relative recovery of cardiac activity generally took place, the initial values being restored after reoxygenation for 10 min, but this was followed by a definite lowering of CO, eventually to half of its initial level before the beginning of asphyxia; this was due not so much to the bradycardia as to the reduction of SV (Fig. 1).

Asphyxia for a period of 7.5 min was found to be critical for restoration of cardiac activity on the resumption of respiration. Contractile activity was not restored in a single animal of the control group, although at the end of the period of asphyxia some restoration of cardiac activity took place, although admittedly, it was weak. In animals of the experimental group, partial restoration both of contractile activity and of the pumping function of the myocardium was observed, although without regaining their original values; just as in the experiments of series I this was due to a reduction of SV, but unlike in series I, the animals of the present series the highest values were recorded toward the end of the period of observation, i.e., toward the 30th minute after the beginning of reperfusion. Combined injection of the opioid receptor antagonist naloxone (500 μg/kg) and of dalargin led to virtually total abolition of the protective effect of dalargin, indicating that its protective action may be mediated by mu- or delta-opioid receptors, a conclusion not contradicted by data in the literature [3, 11].

Progression of hypoxia, i.e., a subsequent increase in its duration to 10 min, was characterized by total inhibition of cardiac activity not only during reoxygenation, but also at the end of the period of hypoxia. Such an increase in the duration of hypoxia was significant even for experimental animals In which, despite infusion of dalargin, the opioid peptide was still unable to limit the hypoxic disturbances, and this naturally was reflected in the level of restoration of the functional parameters during reperfusion, namely 25, 30, and 45% respectively of their initial values, much lower than the corresponding parameters for restoration of cardiac activity after a shorter period of hypoxia. Nevertheless, against the background of dalargin, higher tolerance to hypoxia was observed than in the control, even when its duration was increased. It

TABLE 1. Effect of Dalargin on Functional Parameters of Cardiac Activity during Exposure to High Ca^{2+} Concentrations

Experimental conditions	Aortic flow		Coronary flow	
	Ca^{2+} concentration, mM			
	3	9	3	9
Control (n = 9)	35,2±1,73	0*	15,3±1,37	0,44±0,33*
Dalargin (n = 10)	36,9±2,74	34,5±3,0	15,8±0,78	14,2±1,25
Naloxone (n = 5)	33,4±2,16	0*	17,4±0,75	0*
Naloxone + dalargin (n = 7)	34,1±1,28	0*	14,2±0,37	0,54±0,35*

Legend. *$p < 0.05$ Compared with normal Ca^{2+} concentration in perfusion fluid.

must be recalled that restoration of the contractile function during reperfusion depends not only on the reversibility of the hypoxic disturbances caused by ischemia, but is largely determined by a combination of disorders, affecting many organs and induced by reoxygenation itself [10, 11, 12].

Meanwhile, however, the final stage in the realization of the stressor effect of hypoxia (ischemia – reperfusion), like that of any other form of stress reaction, is an increase in permeability of the cell membranes for Ca^{2+} [6, 7], yet whatever the cause of the increased Ca^{2+} concentration in the cytosol, the excess invariably leads to pathological enhancement of its intracellular regulatory effects. Consequently, the pharmacological maintenance of optimal functioning of the Ca-pump of the sarcoplasmic reticulum and sarcolemma, which is responsible for the rapid and adequate removal of the Ca^{2+} excess from the myofibrils, can considerably limit the development of poststress depression of myocardial pumping function. This hypothesis was tested in experiments to study correction of Ca-homeostasis of the cardiomyocytes by dalargin on a model of hypercalcium perfusion of IPH of intact rats.

The experiments showed that excess of Ca^{2+} in the incubation medium, i.e., overloading of the Ca-pump, on the switching from retrograde to working perfusion by Neely's method led to regular inhibition of all the parameters of the functional state of IPH recorded and, in particular, to the complete disappearance of aortic ejection, evidence of marked inhibition of pumping function, and also to extremely low values of the coronary flow. In the case of combined retrograde perfusion of IPH with hypercalcium solution and with the addition of dalargin (108-8 M) to this perfusion fluid, during subsequent perfusion by Neely's method no appreciable decrease in cardiac activity could be observed (Table 1).

The results indicate the importance, in principle, of the relative maintenance of the Ca-homeostasis of the cardiomyocytes in an experimental model of Ca^{2+} excess in the extracellular space, in the form of a hypercalcium perfusion fluid, by exogenous administration of the synthetic opioid peptide.

The opioid receptor blocker naloxone (200 μg/liter) itself did not limit the overloading of the cardiomyocytes with Ca^{2+}, but abolished this effect virtually completely during combined perfusion with dalargin. This suggests that realization of the protective effect of the synthetic opioid, although only partially, took place through interaction through mu- and (or) delta-opioid binding centers.

In relation to these experiments, one possible explanation of the phenomenon discovered may evidently be the ability of the opioid peptide to reduce Ca^{2+} accumulation during hypercalcium perfusion of IPH, and thereby to prevent the development of a pathological state, in the form of profound disturbances of cardiac function. The possibility cannot be ruled out that it is at this key stage that exogenous peptide administration can break the pathogenetic chain of development of stress-induced damage of whatever etiology, which could partly explain the polyfunctional nature of the opioid peptides in the most widely different extremal states.

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RESPONSE OF THE MAST CELL POPULATION TO INTRATRACHEAL INJECTION OF HEPARIN AND FUCOIDAN

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UDC 612.112.93.014.46:615.273.53].08

KEY WORDS: mast cell; intratracheal injection, heparin, fucoidan

Heparin, a sulfated polysaccharide produced by warm-blooded animals and with a regulatory action on activity of many components of hemostasis, is stored in the cytoplasmic granules of mast cells. To understand the pharmacokinetics of the therapeutic analogs of this biopolymer, the ingestion of substances of polysaccharide nature by mast cells is of great interest. The ability of mast cells to ingest polyanions, introduced into the body, has been demonstrated by light microscopy relative to changes in the number and saturation of the metachromatic staining of the granules by the use of basic dyes [1, 5, 6, 11-13]. It has shown by methods of light microscopy that mast cells can ingest and incorporate granules not only of heparin, but also of other polysaccharides, such as glycogen, inulin, dextran, and carboxymethylcellulose [9, 12]. Depending on structural characteristics, unequal relations would be expected between the internal structures of mast cells and of the polymers entering them. However, visual qualitative assessment of changes in metachromatic staining of the cells was unable to reveal any differences. Mistakes connected with the distribution of granules inside the cell and also with non-specificity of the cationic dyes traditionally used, were unavoidable.

Unlike other basic dyes used in light microscopy, berberine sulfate exhibits a linear relationship between the intensity of its fluorescence and the heparin concentration [10]. The selectivity of binding of berberine sulfate with sulfo groups, under appropriate conditions, enables changes in the content of sulfated polysaccharides in the mast cells to be estimated quantitatively. This has made possible an examination of the known qualitative results of cytochemical analysis at the quantitative level and new information to be obtained on activity of the mast-cell population following administration of various polysaccharides in vivo.

Department of Pharmacology of Hemostasis, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR B. T. Velichkovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 11, pp. 507-509, November, 1991. Original article submitted November 20, 1990.

 0007-4888/91/0011-1612$12.50