Effect of Ligands of Opiate Receptors on Morphofunctional State of the Sympathoadrenal System and Electrical Stability of the Heart in Acute Cold Exposure

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Acute cold exposure (-20°C, 4 h) induces a transient decrease in the ventricular fibrillation threshold without morphological and radionuclide signs of irreversible damage to cardiomyocytes. The agonist of μ-receptors DAGO, which reduces adrenoreactivity of the myocardium, prevents the decrease in the ventricular fibrillation threshold induced by acute cold exposure.

Key Words: *ligands of μ- and δ-receptors; cold; arrhythmias*

Stimulation of the sympathoadrenal system and mobilization of catecholamine (CA), which possess calorigenic properties, represent a defense reaction of the organism to acute cold exposure (ACE) [5,6]. However, excessive CA secretion may damage the myocardium [1]. A direct correlation was established between the incidence of myocardial infarction and fall of outdoor temperature [15]. However, the mechanism of myocardial damage in ACE is poorly understood; moreover, the development of morphofunctional changes in the heart in ACE has not been proved. We have previously shown that opioids prevent the myocardial damage caused by stress and adrenergic agents [2], but the cardioprotective activity of opioid peptides under conditions of ACE has not been studied.

The aim of the present study was to evaluate the role of μ- and δ-opiate receptors (OR) in the regulation of the morphofunctional state of the sympathoadrenal system and electrical stability of the heart in ACE.

MATERIALS AND METHODS

Experiments were carried out on Wistar rats weighing 200-250 g. ACE was modeled by placing the animals in individual cages into refrigerator at -10°C or -20°C for 4 h. In all rats, rectal temperature was measured with an electrothermometer before and after the exposure. In the study of central OR, the agonists of μ-OR DAGO [10] and of δ-OR DADLE [9] (10 μg/rat) and the nonselective antagonist naloxone (20 μg/rat) were injected into the lateral brain ventricle through implanted cannula [3] 1 h prior to ACE. In the study of peripheral OR, DAGO and DADLE in a dose of 0.1 mg/kg and naloxone in a dose of 2 mg/kg were injected intraperitoneally for 5 days before ACE. These doses were chosen in accordance with the data on cardioprotective and antiarrhythmic effects of the opioids [2,3,12].

The rats were decapitated immediately or 24 h after ACE, the heart and adrenals were removed and snap frozen in liquid nitrogen. CA were visualized on cryostate sections with 2% glyoxylic acid [8]. The sections were analyzed cytophotometrically on a LYuMAM-I3 microscope with a FMEL-1 photometer. In adrenal cortex the intensity of the CA fluorescence was measured, while in the heart fluorescent adrenergic nerve endings were counted. The degree of the cold-induced damage to the myocardium was assessed by polarization microscopy of preparations stained with hematoxylin and eosin [7] and by radiometry of the heart after injection of

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^{99m}Tc-pyrophosphate as described elsewhere [13]. In special experimental series the threshold of ventricular fibrillation was measured immediately or 24 h after ACE as described previously [12]. The data were analyzed using the Student's *t* test.

RESULTS

In rats exposed to -10°C for 4 h, rectal temperature did not change, while after a more intense cooling (-20°C) it dropped from 36.8±0.2 to 32.9±0.9°C ($p<0.05$). No signs of myocardial damage were noted immediately and 24 h after ACE. There were solitary contractures of cardiomyocytes which are considered to be reversible changes [7]. Radionuclide imaging revealed no damage to the heart after ACE. For instance, in rats exposed to -20°C for 4 h, the accumulation of ^{99m}Tc-pyrophosphate in the heart did not differ significantly from that in intact animals (2.28±0.35 *vs.* 1.90±0.21 rel. units). However, the threshold of ventricular fibrillation decreased after ACE (-20°C) (Fig. 1) and 24 h later returned to the initial value. Thus, the ACE-induced decrease in the threshold of ventricular fibrillation can be attributed to functional changes in the sarcolemma properties.

What is the mechanism of cardiac electrical instability in ACE? It is known that the decrease in the ventricular fibrillation threshold and even the development of ventricular fibrillation may result from hyperactivation of the sympathoadrenal system [11,14]. Cooling was found to be accompanied by a drop in the CA fluorescence in the adrenals and a decrease in the density of fluorescent adrenergic endings in the heart (Fig. 2). The magnitude of these changes depended on the intensity of cold stress and

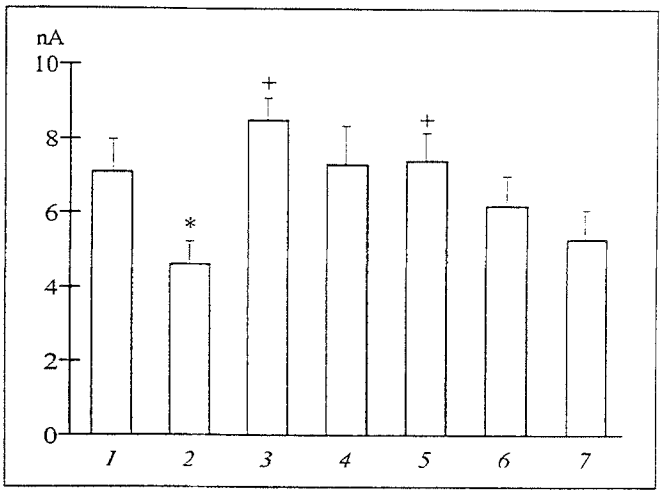

Fig. 1. Effect of the opiate receptor ligands on the ventricular fibrillation threshold in rats after acute cold exposure (ACE). *1*) intact; *2*) ACE; *3*) ACE+24 h; *4*) intact+DAGO; *5*) DAGO+ACE; *6*) intact+DADLE; *7*) DADLE+ACE. *$p<0.05$ compared with *1*; +$p<0.05$ compared with *2*. Here and in Fig. 2: each group consisted of at least 10 animals.

the drop in rectal temperature. According to the published data [1,4] the above-mentioned phenomena attest to activation of the sympathoadrenal system and to a release of CA, but, on the other hand, disturbances in the CA synthesis in ACE cannot be excluded.

Taking these considerations into account, the arising cardiac electrical instability could be corrected with adrenoblockers. However, activation of the sympathoadrenal system under conditions of ACE is an adaptive mechanism [5], and hence, treatment with sympatholytics will inevitably increase mortality of experimental animals caused by hypothermia. These conditions dictate the use of preparations which

TABLE 1. Histochemical Parameters of CA Content in the Adrenal Glands and Heart After ACE in Rats Treated with OR Ligands (*M±m*)

Group	Adrenals, CA fluorescence (arb. units)	Myocardium, density of adrenergic fibers, vol.%
Intact	4.5±0.1	5.4±0.3
Intraperitoneal injection of OR ligands:		
ACE	3.8±0.1***	3.7±0.2***
DAGO+ACE	3.7±0.1***	4.2±0.2**
DADLE+ACE	4.5±0.1**	4.2±0.2**
Naloxone+ACE	3.8±0.1***	2.8±0.2*,+
Intracerebral injection of OR ligands:		
ACE	3.8±0.1***	4.6±0.2*
DAGO+ACE	4.0±0.1**	4.6±0.2*
DADLE+ACE	4.1±0.1*	4.6±0.2*
Naloxone+ACE	3.6±0.1***	4.7±0.2*

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ in comparison with the intact group; +$p<0.01$, +++$p<0.001$ in comparison with the ACE group. Each group consisted of at least 10 rats.

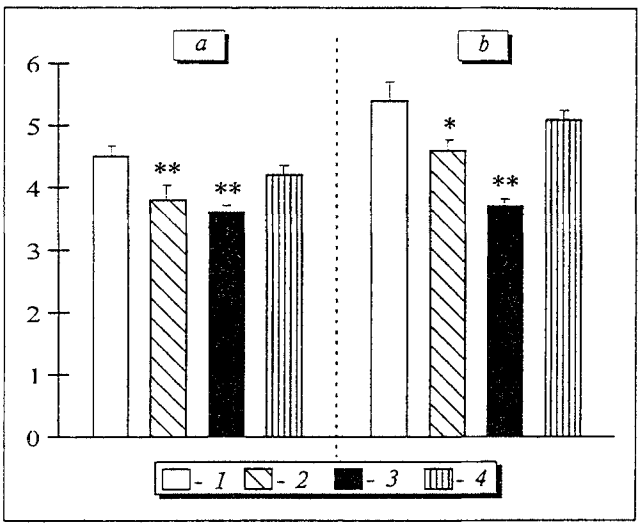

Fig. 2. Histochemical parameters reflecting the catecholamine content in rat adrenals and heart after acute cold exposure (ACE). *a*) catecholamine fluorescence in adrenals (arb. units); *b*) density of adrenergic fibers (vol.%) in the myocardium. *1*) intact; *2*) ACE (-10°C); *3*) ACE (-20°C); *4*) 24 hours after ACE (-20°C). *$p<0.05$, **$p<0.001$ compared with intact rats.

restrict hyperactivation but do not induce chemical sympathectomy. Opioid peptides may be successfully employed as such pharmacological tools, since they have no effect on basal CA secretion but restrict stress-induced activation of the adrenergic system [2].

After intraperitoneal administration, only the δ-agonist DADLE prevented the cold-induced decrease in the adrenal CA content (Table 1). The opiate agonists had no effect on the myocardial level of CA after ACE. The nonselective antagonist naloxone potentated the cold-induced decrease in the myocardial CA content but had no effect on the adrenal content of CA which indicates opiatergic suppression of the CA release from adrenergic endings. The CA-sparing effect of endogenous opioid peptides is primarily related to the activation of myocardial μ- and δ-OR (blocked by naloxone) and adrenal δ-OR (activated by DADLE). The localization of OR mediating the effect of opioid peptides injected intracerebrally remained unclear. None of the preparations injected intracerebrally change the content of CA in the adrenals and heart in ACE (Table 1). These findings suggest that central μ- and δ-OR are not directly involved into realization of the effects of OR ligands in intracerebral application.

Intraperitoneal injection of DAGO, but not DADLE, abolished the cold-induced decrease in the ventricular fibrillation threshold (Fig. 1), whereas in intact animals none of the OR agonists affected this parameter. What is the mechanism of the protective effect of DAGO? On the basis of adrenergic mechanism of decrease in the ventricular fibrillation threshold in ACE, it can be anticipated that DAGO reduces hyperreactivity of the sympathoadrenal system in experimental animals. However, this drug had no effect on the CA release from the adrenals and adrenergic endings in the myocardium. On the other hand, we previously showed that dalargin, an antagonist of peripheral OR, to a greater extent abolishes the cardiotoxic effect of isadrine and prevents the rise of myocardial cAMP [2], thus reducing adrenoreactivity of the heart. All these data suggest that the increase in the ventricular fibrillation threshold induced by DAGO probably results from inhibition of adenylate cyclase in the postsynaptic membrane of cardiomyocytes. However, this hypothesis requires experimental verification.

Thus, our findings suggest that ACE induces transient electrical instability in the heart without morphological and radionuclide sings of irreversible cardiomyocyte damage. Agonists of μ-OR prevent the ACE-induced decrease in the ventricular fibrillation threshold by reducing adrenoreactivity of the myocardium.

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