Opiatergic Mechanisms of the Cardiotropic Effect of Acute Cooling

S. A. Afanas'ev, E. D. Alekseeva, and Yu. B. Lishmanov

UDC 612.014.43.172.225:615.322

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 12, pp. 582-584, December, 1994
Original article submitted January 29, 1994

Changes in myocardial contractility after an acute cold exposure following intracerebroventricular administration of opiate receptor agonists were studied in rat hearts isolated after Langendorff. Cold exposures were carried out individually for each animal in chambers at -10°C for 4 h. Thirty min before being exposed to cold the animals were administered in a brain ventricle 10 μl of μ- or δ-opiate receptor agonists (DAGO or DADLE, respectively). Isolation and perfusion of the hearts were performed directly after the cold exposure was over. The mechanism of reduction of myocardial contractility and coronary flow induced by an acute cold exposure is believed to include stimulation of μ-opiate receptors as one of its main components, and the effect of intracerebral hypertension on hemodynamic parameters is partially mediated through activation of δ-opiate receptors.

Key Words: *cold exposure; isolated heart; contractility*

Today people, particularly those working in the Far North, are frequently exposed to cold, these acute cold exposures (ACE) causing specific changes of the energy metabolism and manifest stress reactions [5]. Some authorities report that stress factors lead to a reduction of the force of contractions and to the development of contracture of an isolated perfused heart [4]. However, the effects of ACE on myocardial contractility and the mechanism of these effects are still poorly studied.

Previously we revealed changes in the activity of the endogenous opiate system in the course of adaptation of an organism to ACE and demonstrated a cardioprotective activity of enkephalins in stress damage to the heart [2,3].

This research was aimed at investigating changes in myocardial contractility after ACE following intracerebroventricular (ICV) administration of μ- and δ-opiate receptor agonists.

Department of Experimental Cardiology, Research Institute of Cardiology, Tomsk Research Center, Russian Academy of Medical Sciences. (Presented by R. S. Karpov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Experiments were carried out with male Wistar rats weighing 250 to 300 g. Five to seven days before the experiment stainless steel hollow cannulas were implanted in the lateral brain ventricles of the animals [11].

Opiate receptor ligands were *ex tempore* dissolved in a 0.9% NaCl solution and 10 μl of this solution were infused at a rate of 5 μl/min. Peptides produced by the Vektor-Bioprodukt Research and Production Amalgamation were used: the δ-opiate receptor agonist [D-Ala2, D-Leu5]-enkephalin (DADLE) [7,12] and the selective agonist of μ-opiate receptors [D-Ala2, N-Met-Phe4, Gly5-ol]-enkephalin (DAGO) [8]. All the peptides were infused in a dose of 20 nmol per rat. The choice of the time of administration and drug dose was based on published data on the dose-dependent analgesic and cardiotropic effects of opioid peptides for ICV administration [6,9,10].

Thirty minutes after ICV administration of peptides the animals were exposed to cold at -10°C for 4 h. Subsequent isolation and 60-min perfusion of

0007-4888/94/0012-1273$12.50

the heart after Langendorff were carried out at the spontaneous frequency of contractions as described previously [1]. Four series of experiments were performed. In two series the animals were administered DAGO or DADLE in the brain ventricles. Experiments with injection of "pure" NaCl were the control for the ICV administration of agonists. Animals of the general control group were exposed to cold without intracerebral injections. The heart contractility curve, its first derivative, and the coronary flow were recorded in the course of the experiment. The amplitude of contractions, rates of contraction and relaxation, myocardial strain, and duration of the cardiac cycle were estimated.

The results were statistically processed using Student's *t* test.

RESULTS

Our previous experiments revealed that exposure of animals to cold markedly changed the contractile activity of the isolated heart and its time course during perfusion. The amplitude of animal heart contractions and coronary flow were reduced immediately after cold exposure by 42 and 20% respectively, *vs.* intact animals ($p<0.05$) and remained virtually unchanged in the course of the subsequent 60-min perfusion. A negligible increase of the strain and a shortening of the cardiac cycle were observed. We unified the method of cooling for all experimental series, and subsequently took the parameters of an isolated heart functioning in the group of "pure" ACE (without intracerebral injections of drugs) as 100%.

The studies showed that the procedure of intracerebral infusion of 10 μl of isotonic NaCl solution itself induced an increase of the amplitude of contractions of isolated hearts of animals exposed to cold (Table 1), this effect persisting during the entire period of perfusion. We associate this reaction with the stimulation of certain brain structures related to hemodynamics regulation. Evidently, such stimulation, which in fact cancels out the cold-induced suppression of contractility, results from an increase of the intracranial pressure in response to the infusion of NaCl into the brain ventricle.

ICV infusion of the δ-opiate receptor agonist DADLE leads to an increase in the contractility of the isolated heart in comparison with the group of animals exposed to cold but administered no intracerebral injections. Noteworthy is a clear trend toward an increase of the amplitude of contractions as compared to animals injected NaCl, this indicating a possible role of the modulation of hemodynamic effects of ACE, which is specifically related to the δ-opiate receptors.

This hypothesis was confirmed in experiments with ICV administration of the selective μ-agonist DAGO, which had a fundamentally different effect on the myocardial contractility of animals exposed to cold. As is seen from Table 1, stimulation of the μ- opiate receptors was associated with a reduction of the inotropic function of the myocardium in comparison with both the DADLE series and the effect of NaCl.

In assessing the diastolic function of the heart by its tension in the course of 60-min perfusion, we revealed that ICV infusion of NaCl to cooled rats led to a rapid increase of contractures, by 120% on an average, *vs.* the group of ACE controls (Table 1). Such effects were not recorded after ICV injection of DAGO or DADLE. Moreover, these agents reliably reduced the contractile tension of the isolated heart. Hence, it may be assumed that activation of both δ- and μ-opiate receptors of the brain is conducive to preventing ACE-induced diastolic dysfunction of the heart.

Activation of myocardial contractions observed in experiments with infusion of NaCl was not paralleled by statistically reliable changes in the rates of contraction and relaxation in the course of perfusion. A high basal level of these parameters in comparison with the ACE control group was in line with the above data on the positive inotropic effect of ICV injection of NaCl. The initial values of the rate of contraction and relaxation in rats exposed to cold after ICV injection of DADLE did not reliably differ from those in the NaCl series, but by the time of perfusion cessation they had appreciably increased. In contrast to this, an infusion of DAGO was associated with a reduction of the rates of contraction and relaxation of the myocardium at all perfusion times tested.

The high rates observed for ICV infusion of NaCl and DADLE correlated well with the manifest positive inotropic effect of these injections. Since DAGO and DADLE did not differ in their effects on development of heart contractures under conditions of spontaneous heartbeat frequency, the detected differences in their effects on the time course of the rate of contractions may be considered to result from variously directed changes in the chronotropic function of the heart. An injection of DAGO caused a reliable prolongation, and DADLE a reliable shortening of the cardiac cycle (Table 1).

We have already mentioned that ACE was associated with a noticeable reduction of the coronary flow. ICV injection of NaCl under the said conditions did not appreciably influence this parameter, and infusions of opioid peptides, irrespective of the inotropic reaction induced by them,

TABLE 1. Effect of Intracerebral Injections and Acute Cooling of Rats on the Contractile Activity of the Isolated Heart of These Animals in % to the Values in Controls Taken as 100% ($M \pm m$)

Parameter	Series	Time of recording of parameters, min				
		adaptation	15	30	45	60
Amplitude						
	control	100±9	100±5	100±5	100±5	100±9
	NaCl	193±31*	178±34*	156±32*	197±34*	212±35*
	DAGO	134±13°	100±11°	81±8°	80±1°	101±9°
	DADLE	213±16*	222±10*	196±18*	230±18*	262±29*
Strain						
	control	100±16	100±38	100±36	100±31	100±21
	NaCl	93±17	151±25	221±35*	169±23	157±23
	DAGO	20±13*°	33±17*°	42±31°	68±23°	93±19°
	DADLE	53±9*°	69±5°	99±30°	92±14°	103±14°
Rate of contractions						
	control	100±14	100±11	100±11	100±5	100±8
	NaCl	184±27*	191±37*	161±30*	176±33	189±37*
	DAGO	139±1°	97±11°	78±8°	74±9°	88±8°
	DADLE	187±11*	184±16*	190±13*	249±26*°	257±27*°
Rate of relaxation						
	control	100±21	100±14	100±15	100±8	100±9
	NaCl	206±18*	205±20*	172±21*	194±22*	203±22*
	DAGO	118±14°	81±13°	57±7*°	48±7*°	52±7*°
	DADLE	195±27*	214±21*	217±21*°	269±27*°	307±25*°
Coronary flow						
	control	100±16	100±18	100±16	100±17	100±17
	NaCl	81±11	77±12	72±10	68±1	78±12
	DAGO	32±3*°	30±5*°	27±5*°	19±3*°	20±3*°
	DADLE	63±7*	61±5*	60±10*	59±8*	67±9*
Cycle duration						
	control	100±7	100±2	100±5	100±5	100±6
	NaCl	113±3	108±5	108±4	112±3	114±3
	DAGO	100±4	106±6	112±9	128±8*°	142±5*°
	DADLE	67±12*°	69±13*°	64±10*°	69±10*°	72±11*°

Note. An asterisk shows reliable differences *vs.* the control; a circle denotes reliable differences from the NaCl series.

were conducive to a reliable reduction in the volume of coronary perfusion.

It is noteworthy that the reduction of the coronary flow under the effect of the μ-agonist DAGO was observed in the presence of progressive bradycardia. This suggests a direct effect of opioid peptides on the smooth muscles of the vessels as a mechanism of their effect on the coronary flow.

It is possible that the mechanism of ACE-induced reduction of myocardial contractility and coronary flow includes as one of its major components stimulation of the μ-opiate receptors, and the effects of intracerebral hypertension are partially mediated through activation of the δ-opiate receptors.

This research was carried out within the framework of project № 93-04-20357 of the Russian Foundation for Basic Research.

REFERENCES

1. B. I. Laptev, S. A. Afanas'ev, V. D. Prokop'eva, *et al.*, *Ukr. Fiziol. Zh.*, № 4, 22 (1987).
2. Yu. B. Lishmanov, *Byull. Eksp. Biol. Med.*, **102**, № 9, 271-272 (1986).
3. Yu. B. Lishmanov, L. N. Maslov, I. G. Khaliulin, and N. A. Barbarash, *Vestn. Ross. Acad. Med. Nauk*, № 3, 5-8 (1992).
4. F. Z. Meerson and M. G. Pshennikova, *Adaptation to Stress Situations and Physical Exercise* [in Russian], Moscow (1988).
5. H. Selye, *Story of the Adaptation Syndrome*, Acta, Montreal (1952).
6. B. D. Appelbaum and S. G. Holtzman, *Brain Res.*, **377**, 330-336 (1986).
7. G. Gacel, M.-C. Fournie-Zaluski, and B. P. Roques, *FEBS Lett.*, **118**, № 2, 245-247 (1980).
8. B. K. Handa, A. C. Lane, J. A. H. Lord, *et al.*, *Europ. J. Pharmacol.*, **70**, 531-540 (1981).
9. J. W. Holaday, *Peptides*, **3**, 1023-1029 (1982).
10. M. Laubie and H. Schmitt, *Europ. J. Pharmacol.*, **71**, 401-409 (1981).