Antiarrhythmic Activity of the Antiopioid Peptide Nociceptin and its Effect on the Fast Na^+ Channels in Cardiomyocytes

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 126, No. 12, pp. 655-657, December, 1998
Original article submitted January 15, 1998

The antiopioid peptide nociceptin does not affect heart resistance to the proarrhythmic effect of epinephrine or calcium chloride, but it produces an antiarrhythmic effect in the aconitine arrhythmia model. Nociceptin prolongs the QRS interval and does not affect heart rate and duration of the RQ interval. The antiarrhythmic effect of nociceptin is not related to changes in the tone of the autonomic nervous system. Nociceptin is supposed to block fast Na^+ channels in cardiomyocytes.

Key Words: *nociceptin; arrhythmias; fast Na^+ channels*

Endogenous opioid neuropeptides play an important role in the regulation of cardiovascular system [2]. Stimulation of opiate receptors may enhance cardiac tolerance to proarrhythmic influences [3,7,9]. Functional antagonists of opioid peptides (cholecystokinin, FMRF-peptide, neuropeptide FF *etc.*) were found in humans and animals. They do not directly affect the opiate receptors, but produce effects that are opposite to those of opioids on the cardiovascular system [4]. The family of "antiopioids" was recently enlarged by another peptide, nociceptin (NC or orphanin FQ) [8,10]. The specific opioid receptor-like (ORL) receptors for this peptides are linked to adenylate cyclase via G-proteins [8,10]. The interaction of NC with adenylate cyclase inhibits the synthesis of cAMP [8, 10], which is an endogenous proarrhythmic factor [6]. It is probable that NC-induced decrease of cAMP level enhances the electrical stability of the heart. However, the opioid peptides are also known to produce an antiarrhythmic effect [2]. To resolve this contradiction, we studied the effect of NC on the heart tolerance to proarrhythmic factors.

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MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 180-200 g in which arrhythmias were provoked by intravenous epinephrine (100 μg/kg) or $CaCl_2$ (100 mg/kg) under light ether anesthesia. The ECG was recorded in the second standard lead (V_2) during the first 5 minutes postinjection. It was processed to obtain the incidence rates of ventricular extrasystoles, ventricular tachycardia, and ventricular fibrillation.

In one experimental series arrhythmias were produced by intravenous aconitine (50 μg/kg, Sigma). To this end, aconitine (1 mg) was suspended in Tween-80 (20 ml) and mixed with 20 ml 0.9% NaCl. The suspension was administered in a dose of 1 ml/kg. The latent period from injection to the appearance of ventricular arrhythmia was documented.

The agonist of ORL receptors NC (Phe-Gly-Gly-Phe-Thr-Gly- Ala-Arg-Lys-Ser-Ala-Arg- Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asp-Cln), was synthesized at the Institute of Molecular Pharmacology (Berlin). It was dissolved *ex tempore* in isotonic NaCl solution and injected intravenously (0.1-0.4 mg/kg) 10 min prior to a proarrhythmic agent. In one experimental series the rats were injected intravenously with hexamethonium (10 mg/kg, Sigma), a blocker of peripheral autonomic ganglia.

0007-4888/98/0012-1224$20.00

The ECG recording and processing were performed with the help of a UBF4-03 amplifier and original software. The results were statistically analyzed using Student's t and χ^2 tests.

RESULTS

Nociceptin, a selective agonist for ORL receptors, did not affect cardiac tolerance to the proarrhythmic effect of epinephrine and $CaCl_2$ (Table 1), although it prolonged in a dose-dependent manner the latency of rhythm disturbances induced by aconitine (Fig. 1). At 0.1 mg/kg it did not affect this latency, which increased by 30% and almost 2-fold after 0.2 mg/kg or 0.4 mg/kg NC, respectively.

The antiarrhythmic effect of NC was not related to changes in the autonomic nervous system tone because the ganglioblocker hexamethonium did not affect the antiarrhythmic effect of NC (Fig. 1). Presumably, NC directly affects the myocardium; however, more evidence about the NC target should be obtained in experiments with isolated perfused heart.

Based on the data about NC ability to selectively increase the heart tolerance to proarrhythmic effect of aconitine without affecting the incidence of epinephrine- or $CaCl_2$-induced arrhythmias, one can propose a mechanism underlying the pharmacological effect of NC. The proarrhythmic effect of $CaCl_2$ is caused by a direct extra inflow of Ca^{2+} to the cytoplasm, while the potency of catecholamines to induce arrhythmias is related to increased cAMP production with subsequent increase of free Ca^{2+} in cardiomyocytes [6,12]. The aconitine-induced arrhythmia results from an increase in intracellular Ca^{2+} caused by enhanced Na^+/Ca^{2+} exchange [5] in response to the rise of intracellular Na^+ that results from the slowing down of inactivation of fast Na^+ channels [1].

The antiarrhythmic effect of NC may be mediated via the inhibition of Na^+/Ca^{2+} exchange or block of the fast Na^+ channels. The effect of NC on transmembrane Ca^{2+} transport or on the level of intracellular cAMP should be excluded, since the examined peptide did not affect the epinephrine- or $CaCl_2$-induced arrhythmias.

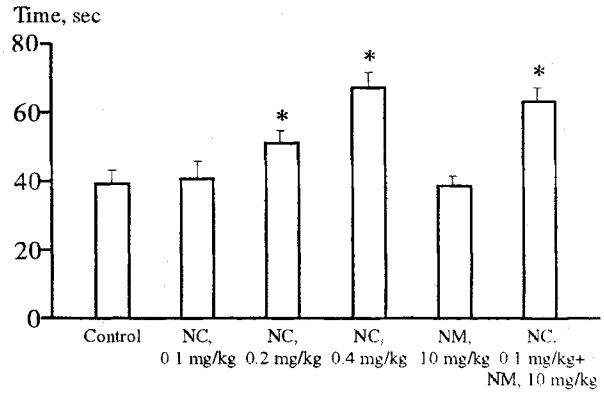

Fig. 1. Effect of nociceptin (NC) and hexamethonium (HM) on latency of aconitine-produced arrhythmias. $p<0.001$ in comparison with control.

The probability that sarcolemmic Na^+ channels in cardiomyocytes are involved in the antiarrhythmic performance of NC is evidenced by analysis of the effect of NC on the ECG in intact animals. Five minutes postinjection of 0.2 mg/kg NC the *QRS* complex expanded from 28.4±0.8 to 41.1±0.9 msec (by 44%, $p<0.001$). This prolongation was maintained to the 10th minute of observation. Simultaneously, a highly significant ($p<0.001$) increase in the *QT* interval duration was observed on the 5th min (from 49.6±1.0 to 58.4±1.1 msec, or by 18%) and the 10th min (by 16%). There were not statistically significant changes in the *PQ* interval duration and in heart rate.

The pronounced effect of NC on the *QRS* duration in combination with a slight slowing down of the repolarization phase (*QT* interval) and lack of changes in automatism and conductance is similar to the effects of the fast Na^+ channel blockers quinidine, disopyramide, ethacisine, and allapinin [11]. Our findings imply the involvement of fast Na^+ channels in the pharmacological effect of NC.

TABLE 1. Effect of NC on Appearance of Epinephrine- and $CaCl_2$-Induced Arrhythmias

Group of rats	Ventricular extrasystoles	Ventricular tachycardia	Ventricular fibrillation
	number of rats (%)		
Epinephrine-induced arrhythmias			
Control (n=20)	13 (65)	12 (60)	2 (10)
NC, 0.4 mg/kg (n=12)	10 (83)	10 (83)	2 (17)
$CaCl_2$-induced arrhythmias			
Control (n=14)	9 (64)	11 (79)	4 (29)
NC, 0.4 mg/kg (n=14)	10 (71)	11 (79)	5 (36)

Our results point to the ability of the agonists of peripheral ORL receptors to enhance cardiac tolerance to proarrhythmic interventions. The most probable mechanism of this phenomenon is the inhibition of Na^+/Ca^{2+} exchange or block of the fast sarcolemmic Na^+ channels in cardiomyocytes.

We are grateful to Dr. Erika Albrecht (Institute of Molecular Pharmacology, Berlin) for NC and Dr. D. S. Ugdyzhekova for technical assistance.

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