Possible Mechanism Underlying the Antiarrhythmic Effect of Peptides Nociceptin and DALDA on the Heart

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During aconitine-induced arrhythmias the antiarrhythmic effect of DALDA (Tyr-D-Arg-Phe-Lys-NH_2) and nociceptin (orphanin FQ) administered intravenously depended on activation of nitric oxide synthase. K_{ATP} channels were not involved in the realization of this effect. Endogenous prostanoids played a minor role in the antiarrhythmic effect of nociceptin and did not contribute to the protective influence of DALDA. The antiarrhythmic effect of orphanin FQ administered intravenously did not depend on functional activity of the autonomic nervous system. However, the effect of orphanin FQ after intracerebroventricular infusion was determined by changes in the state of this system.

Key Words: *arrhythmias; nociceptin; DALDA*

Our previous studies of aconitine-induced arrhythmias showed that stimulation of μ-opiate and opioid-like receptors (OR and ORL1, respectively) with intravenous administration of selective agonists DALDA and nociceptin (orphanin FQ) increases the resistance to cardiac arrhythmias [3,4]. However, the mechanism underlying this effect of peptides remained unknown. The arrhythmogenic effect of aconitine is associated with impaired inactivation of fast Na^+ channels [1]. It can be hypothesized that the antiarrhythmic effect of μ-OR and ORL1 agonists results from blockade of Na^+ current. However, it is unlikely that DALDA and orphanin FQ directly interact with Na^+ channel proteins. μ-Conotoxin GIIA is the only selective peptide blocker of Na^+ channels isolated from venom of marine snails *Conus geographus* [12]. Moreover, there are no data on toxic activity of nociceptin or peptide agonists of μ-OR. However, selective blockers of Na^+ current tetrodotoxin and conotoxin possess high toxicity. Various intracellular messengers, including nitric oxide (NO), are probably involved in signal transduction from μ-OR and ORL1 to Na^+ channels. Previous studies revealed a relationship between NO synthesis and resistance to occlusion-reperfusion arrhythmias [13]. Published data show that the vasodilator effect of peptide μ-OR agonists and nociceptin results from stimulation of NO synthase [6,8]. The vasodilator properties of orphanin FQ and the antiarrhythmic effect of peptide μ-OR agonists are associated with activation of ATP-dependent K^+ channels (K_{ATP} channels) [5]. We hypothesized that an increase in the resistance to aconitine-induced arrhythmias mediated by μ-OR and ORL1 is related to activation of NO synthase or K_{ATP} channels.

Our previous studies showed that the cardioprotective effect of peptide agonists is associated with the synthesis of endogenous prostanoids [2]. These compounds can mediate the antiarrhythmic action of opiates and orphanin FQ. It remains unclear whether the antiarrhythmic effect of nociceptin results from activation of peripheral or central ORL1.

Here we studied the role of cyclooxygenase, NO synthase, and K_{ATP} channels in the antiarrhythmic effect of DALDA (peptide agonist of μ-OR) and nociceptin during aconitine-induced arrhythmias and eva-

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luated the contribution of central and peripheral nociceptin receptors in the regulation of antiarrhythmic activity in the myocardium.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 250-300 g and narcotized with diethyl ether. Cardiac arrhythmias were produced by intravenous administration of 100 μg/kg aconitine. ECG was recorded (lead II) on an UBF4-03 biopotential amplifier coupled to IBM 486 computer and processed using original application software. The interval between aconitine administration and appearance of ventricular tachycardia or fibrillation (latency) was determined.

Tyr-D-Arg-Phe-Lys-NH_2 (DALDA) served as a selective agonist of μ-OR. Published data show that this peptide administered intravenously cannot cross the blood-brain barrier (BBB) [14]. DALDA was injected intravenously in a dose of 0.1 mg/kg. In this dose peptide agonists exhibited high antiarrhythmic activity [3]. Nociceptin (Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asp-Gln) was *ex tempore* dissolved in 0.9% NaCl and injected intravenously in a dose of 0.4 mg/kg 10 min before aconitine administration. The dose of orphanin FQ was selected taking into account published data on its antiarrhythmic effect [4]. Indomethacin (cyclooxygenase inhibitor) was administered in a dose of 5 mg/kg 30 min before injection of aconitine [2]. Indomethacin was dissolved in ethanol and physiological saline to a final concentration of 5 mg/ml. The concentration of ethanol did not exceed 0.1% [2]. The selective blocker of K_{ATP} channels glybenclamide was injected intravenously in a dose of 0.3 mg/kg 45 min before aconitine administration [15]. Glybenclamide was dissolved in 45% aqueous solution of 2-hydroxypropyl-β-cyclodextrin. The NO synthase inhibitor N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) was injected intravenously in a dose of 50 mg/kg 25 min before aconitine administration [6]. Hexamethonium was injected intravenously in a dose of 10 mg/kg 15 min before aconitine administration to evaluate the contribution of the autonomic nervous system into the antiarrhythmic effect of nociceptin.

Experiments with intracerebroventricular administration of orphanin FQ were performed on male Wistar rats weighing 250-300 g. A stainless steel hollow cannula was implanted into the lateral cerebral ventricle and fixed on the skull with stomatological cement phosphate 5-7 days before induction of arrhythmias. Surgery was performed under barbamyl anesthesia (50 mg/kg intraperitoneally) using a SEZh-5 stereotactic device (Konstruktor; stereotactic coordinates: AP-1.5 mm, L+2.0 mm, V-3.5 mm) [13]. Methylene blue in a dose of 5 μl was injected intracerebroventricularly before decapitation to verify localization of the cannula. Orphanin FQ was *ex tempore* dissolved in 0.9% NaCl and infused (10 μl, 5 μl/min) 30 min before administration of 36 μg aconitine.

Nociceptin was synthesized at the Institute of Molecular Pharmacology (Germany). DALDA was obtained from the Multiple Peptide Systems Company (USA). Aconitine, hexamethonium, indomethacin, L-NAME, and 2-hydroxypropyl-β-cyclodextrin were obtained from the Sigma-RBI Company (USA).

Control animals received intravenous or intracerebroventricular injections of physiological saline. The results were analyzed by Student's *t* test.

RESULTS

In control animals the latency of ventricular arrhythmias after aconitine administration was 39 sec (Fig. 1). DALDA administered intravenously increased the latency by 3 times. The K_{ATP} channel blocker glybenclamide and cyclooxygenase inhibitor indomethacin did not modulate the antiarrhythmic effect of DALDA. It should be emphasized that indomethacin had no effect on arrhythmogenic activity of aconitine, while glybenclamide increased the latency by 16%. Blockade of NO synthase with L-NAME attenuated, but did not abolish the antiarrhythmic effect of DALDA. L-NAME alone did not modulate the effect of aconitine.

Our results indicate that prostanoids are not involved into the antiarrhythmic effect of DALDA. During coronary occlusion and reperfusion the antiarrhythmic

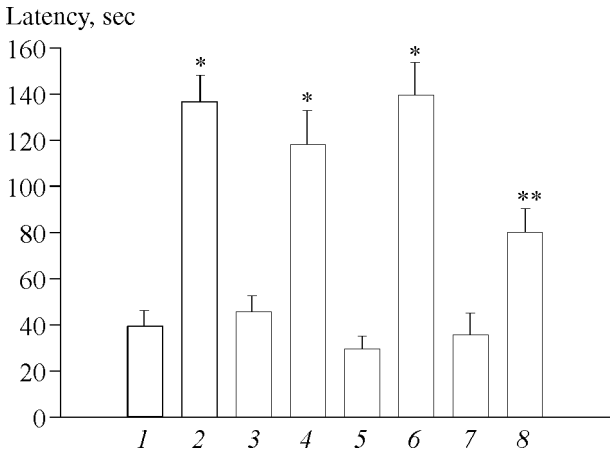

Fig. 1. Effects of glybenclamide, indomethacin, and L-NAME on a DALDA-mediated increase in the average latency of aconitine-induced arrhythmias. Each experimental group included 16 animals. The control group included 20 animals. Control (*1*), DALDA (0.1 mg/kg, *2*), glybenclamide (0.3 mg/kg, *3*), glybenclamide and DALDA (*4*), indomethacin (5 mg/kg, *5*), indomethacin and DALDA (*6*), L-NAME (50 mg/kg, *7*), L-NAME and DALDA (*8*). *$p<0.01$ and **$p<0.05$ compared to the control.

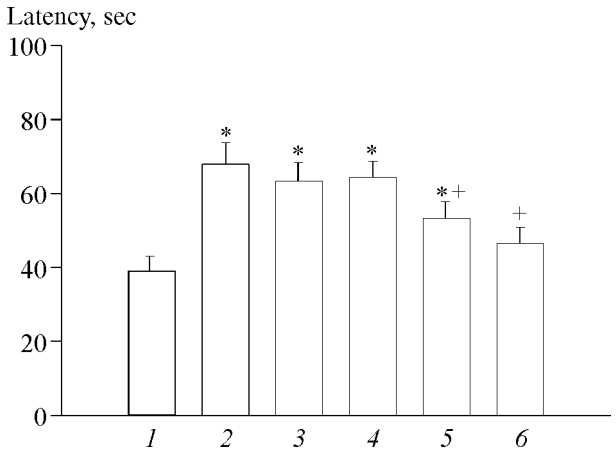

Fig. 2. Effects of hexamethonium, glybenclamide, indomethacin, and L-NAME on a nociceptin-mediated increase in the average latency of aconitine-induced arrhythmias. Each experimental group included 16 animals. The control group included 20 animals. Control (*1*), nociceptin (0.4 mg/kg, *2*), hexamethonium (10 mg/kg) and nociceptin (0.4 mg/kg, *3*), glybenclamide (0.3 mg/kg) and nociceptin (0.4 mg/kg, *4*), indomethacin (5 mg/kg) and nociceptin (0.4 mg/kg, *5*), L-NAME (50 mg/kg) and nociceptin (0.4 mg/kg, *6*). **p*<0.001 compared to the control; ^{+}p<0.01 compared to nociceptin.

effect of DALDA depended on activation of K_{ATP} channels. Therefore, the increase in heart resistance to aconitine after administration of this peptide was not realized via K_{ATP} channels. NO synthase plays an important role in the antiarrhythmic effect of DALDA during aconitine-induced arrhythmias. However, NO synthase blockade did not completely abolish the effect of this peptide. These findings indicate that the increase in the resistance to aconitine-induced cardiac arrhythmias after administration of DALDA is realized not only via the NO synthase system, but also via another unknown mechanism. In our experiments we

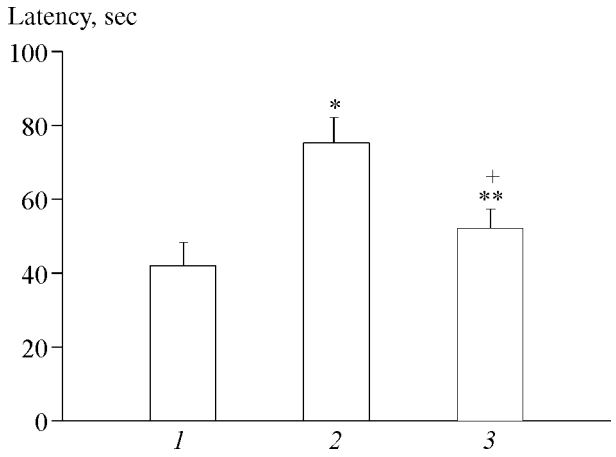

Fig. 3. Effect of hexamethonium on the antiarrhythmic effect of nociceptin infused intracerebroventricularly during aconitine-induced arrhythmias: control (*n*=16, *1*), nociceptin (36 μg, *n*=10, *2*), hexamethonium (10 mg/kg) and nociceptin (36 μg, *n*=14, *3*). **p*<0.001 and ***p*<0.05 compared to the control; ^{+}p<0.05 compared to nociceptin.

did not use OR blockers. However, it can be suggested that the ability of DALDA to increase the latency of aconitine-induced arrhythmias is related to activation of peripheral μ-OR. Published data show that DALDA is highly selective for μ-OR [10] and does not cross BBB after intravenous administration [14]. Our previous experiments demonstrated that the antiarrhythmic effect of DALDA during coronary occlusion and reperfusion is associated with activation of peripheral μ-OR.

Nociceptin promoted the increase in the resistance to aconitine-induced cardiac arrhythmias (Fig. 2). However, antiarrhythmic activity of orphanin FQ was lower than that of DALDA. Nociceptin increased the latency of arrhythmias only by 65%. The blocker of peripheral autonomic ganglia hexamethonium had no effect on the latency of arrhythmias and did not modulate cardiac sensitivity to aconitine. Therefore, the autonomic nervous system does not play a role in the antiarrhythmic effect of nociceptin administered intravenously during aconitine-induced arrhythmias. Intracerebroventricular infusion of nociceptin increased the latency of aconitine-induced arrhythmias by 1.8 times (Fig. 3). This effect was abolished by hexamethonium. Thus, the antiarrhythmic effect of nociceptin can result from changes in functional activity of the autonomic nervous system. Intracerebroventricular infusion of orphanin FQ enhances tonic activity of the vagus nerve and attenuates adrenergic stimulation of the heart [7]. Probably, the increase in the resistance to aconitine was associated with the rise in vagal tone and decrease in tonic activity of the sympathetic nervous system produced by nociceptin.

K_{ATP} channel blockade with glybenclamide did not modulate the antiarrhythmic effect of orphanin FQ administered intravenously (Fig. 2). Therefore, K_{ATP} channels play an insignificant role in the antiarrhythmic effect of nociceptin. The cyclooxygenase inhibitor indomethacin attenuated (by 22%), but did not abolish the antiarrhythmic effect of nociceptin. However, NO synthase blockade with L-NAME completely abolished the antiarrhythmic effect of orphanin FQ. Thus, NO and NO synthase play a key role in the antiarrhythmic effect of nociceptin. Prostanoids play little role in a nociceptin-produced increase in heart resistance to aconitine.

Orphanin FQ is a selective agonist of ORL1 [9]. Antiarrhythmic activity of nociceptin after intravenous administration is probably associated with activation of peripheral ORL1. BBB is low permeable for most pentapeptides and hexapeptides. There is little likelihood that intravenously injected orphanin FQ enters the brain tissue. At first glance it would seem that our results contradict this assumption. Intracerebroventricular infusion of nociceptin promoted the increase in the resistance to aconitine-induced arrhythmias. How-

ever, this effect disappeared after blockade of peripheral autonomic ganglia with hexamethonium. The antiarrhythmic effect of intravenously injected orphanin FQ was observed after "pharmacological denervation" of the heart with hexamethonium. Therefore, activation of central and peripheral ORL1 can be accompanied by an increase in the resistance to aconitine-induced arrhythmias. In the former case, the antiarrhythmic effect of nociceptin resulted from changes in functional activity of the autonomic nervous system. In the latter case, this effect depended on NO synthase activity.

Our results indicate that the antiarrhythmic effect of selective agonists of μ-OR and ORL1 administered intravenously during aconitine-induced arrhythmias depends on activation of NO synthase. K_{ATP} channels are not involved in the realization of this effect. Endogenous prostanoids play little role in the antiarrhythmic effect of nociceptin and do not contribute to the protective influence of DALDA. The antiarrhythmic effect of orphanin FQ administered intravenously does not depend on functional activity of the autonomic nervous system. However, the action of orphanin FQ after intracerebroventricular infusion is determined by changes in the state of this system.

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