Negative Inotropic and Chronotropic Effects of δ-Opioid Receptor Antagonists Are Mediated via Non-Opioid Receptors

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Ten-minute perfusion of intact isolated rat heart with Krebs—Henseleit solution containing δ-opioid receptor agonists (DPDPE, (-)-TAN-67) or δ-opioid receptor antagonists (naltrindole, TIPP[ψ], ICI 174,864) at a final concentration of 0.1 mg/liter decreased HR, blood pressure in the left ventricle, and the rates of myocardial contraction and relaxation. Intravenous injection of δ-agonists (DPDPE, (-)-TAN-67, deltorphin II) or δ-antagonists (naltrindole, TIPP[ψ], ICI 174,864) decreased HR in narcotized rats. Naloxone and naltrexone produced no effect on contractility and HR both *in vivo* and *in vitro*. Preliminary injection of naloxone and naltrexone did not prevent the negative chronotropic effect of ICI 174,864 *in vitro*. The negative inotropic and chronotropic effects of δ-opioid receptor antagonists are mediated by unknown non-opioid receptors in the heart.

Key Words: *δ-opioid receptor antagonists; cardiac rhythm; isolated perfused heart*

In early 1990, researchers cloned four types of opioid receptors (OR) and deciphered their molecular structure. These OR receptors were OR_1 (μ), OR_2 (δ), OR_3 (k), and OR_4 (ORL1) [3,4,8]. While cloning of new receptors is in progress, the possibility exists that some ligands are erroneously assumed selective. It is demonstrated that naloxone and naltrexone are neutral OR ligands, which *per se* produce no effect on the cell [7]. Selective δ-OR antagonists can exhibit intrinsic negative activity *in vitro* (*i.e.* produce effects opposite to that of agonists) [7]. However, it is not clear whether δ-antagonists produce similar effect on isolated perfused heart, and how they affect the heart *in vivo* [2]. Opioid peptides were shown to interact with so-called "non-opioid receptors of opioid peptides" [1,5,9]. There are no similar data on the interaction of OR antagonists with non-opioid receptors, but this phenomenon cannot be excluded.

Our aim was to assess the receptor nature of inotropic and chronotropic effects of δ-OR antagonists on the heart *in vivo* and *in vitro*.

MATERIALS AND METHODS

The experiments were performed on rat heart *in vivo* and *in vitro*. *In vitro* study was carried out on male Wistar rats weighing 300-350 g. After thoracotomy, the heart was rapidly excised from the thorax and placed in cold (4°C) Krebs—Henseleit solution. When spontaneous beats stopped, the ascending arch of the aorta was cannulated and isotonic physiological saline was infused. The retrograde Langendorff perfusion of the heart was per-

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formed using standard Krebs—Henseleit solution. Myocardial contractility was recorded when the heart was perfused under a constant pressure of 52 mm Hg. The contraction parameters were recorded with an electromanometer connected to a latex balloon inserted into the left ventricle.

The following parameters were recorded: HR (min^{-1}), left ventricular pressure difference (LVPD, mm Hg); maximum contraction rate (mm Hg/sec); and maximum relaxation rate (mm Hg/sec). LVPD was calculated as the difference between systolic and diastolic pressure. After the end of adaptation (20 min) to normoxic perfusion, one of the test ligands was added to the Krebs—Henseleit solution to a final concentration of 0.1 mg/liter, and the normoxic perfusion was continued for 10 min. The contraction parameters were determined before application of the ligand and immediately after termination of 10-min perfusion of the heart with ligand-containing solution.

The study used selective agonists of δ-OR DPDPE (H-Tyr-D-Pen-Gly-Phe-D-Pen-OH) and (-)-TAN-67 ((-)-2-methyl-4aa-(3-hydoxyphenyl)-1,2,3,4, 4a,5,12,12, 12aα-octahydroquinolino[2,3,3-g]isoquinoline dihydrobromide), selective δ-antagonists TIPP[ψ] (H-Tyr-Ticψ)[CH_2NH]Phe-Phe-OH), ICI 174,864 (N,N-Diallyl-Tyr-Aib-Aib-Phe-Leu-OH), selective δ-antagonist naltrindole, nonselective OR antagonists naloxone and naltrexone. *In vivo* study used OR ligands in the following doses: 0.09 mg/kg DPDPE (138 nmol/kg), 0.12 mg/kg deltorphin II (153 nmol/kg), 0.08 mg/kg (-)-TAN-67 (158 nmol/kg), 0.2 (254 nmol/kg) and 0.5 mg/kg (635 nmol/kg) ICI 174,864, 0.5 mg/kg TIPP[ψ] (770 nmol/kg), and 1 mg/kg naltrindole (2200 nmol/kg).

The parameters of these preparations were described elsewhere [4,8]. OR ligands were dissolved in physiological saline before the experiment. The doses were chosen on the basis of published [12, 14] and own [11] data.

Peptide OR ligands were synthesized by Multiple Peptide Systems Ltd. Naltrindole, naloxone, and naltrexone were from Tocris Cookson Ltd, and chloralose was from Sigma. δ-OR agonist (-)-TAN-67 was kindly gifted by Toray Industries Ltd. Krebs—Henseleit solution consisted of the agents from ICN Biomedicals.

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

RESULTS

Normoxic 10-min perfusion of intact myocardium with physiological saline containing δ_1-agonist (DPDPE or (-)-TAN-67) or δ-antagonist (naltrindole, TIPP[ψ], or ICI 174,864) decreased HR. On average, the agonists to δ_1-OR and antagonists to δ-OR decreased HR by 30% and 2-fold, respectively. These agents added to the perfusion solution in a final dose of 0.1 mg/liter 2-fold decreased LVPD. In addition, they reduce the minimum contraction and relaxation rates. All these agents are selective ligands of δ-OR and produce negative inotropic and chronotropic effects on the isolated heart. By contrast, naloxone (275 nM) had no effect on HR and contraction parameters of the heart.

The effect of δ-antagonists (naltrindole, TIPP[ψ], or ICI 174,864) and δ-agonists (DPDPE and (-)-TAN-67) in a dose of 0.1 mg/liter on the pumping function of the isolated heart was similar.

A pronounced drop in HR was observed on minute 15 after intravenous injection of selective δ-OR agonists DPDPE, deltorphin II, or (-)-TAN-67 (Fig. 1). The most potent negative chronotropic effect was produced by deltorphin II and (-)-TAN-67 (Fig. 1). These agents decreased HR by 13%. On minute 10 postinjection, δ-OR antagonists naltrindole and ICI-174,864 produced sustained bradycardia. Injection of TIPP[ψ] decreased HR, but the effect was observed only on minute 20 postinjection. The most pronounced decrease in HR (13%) was produced by ICI-174,864. Nonselective OR antagonists naloxone and naltrexone (0.05 mg/kg) had no effect on HR. There were no significant changes of *PQ*, *QRS*, *QT*, and *QT* intervals in ECG. The effect of 0.9% NaCl on ECG can be excluded, since no changes in HR and ECG were recorded when physiological saline without OR ligands was injected.

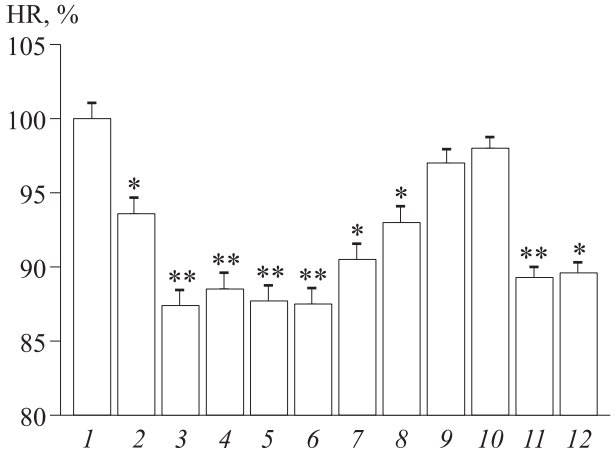

Fig. 1. Effect of δ-OR ligands on HR in vivo. *1*) initial value (control); *2*) DPDPE (0.09 mg/kg); *3*) (-)-TAN-67 (0.08 mg/kg); *4*) deltorphin II (0.12 mg/kg); *5*) ICI 174,864 (0.5 mg/kg); *6*) ICI 174,864 (0.2 mg/kg); *7*) naltrindole (1 mg/kg); *8*) TIPP[ψ] (0.5 mg/kg); *9*) naloxone (0.05 mg/kg); *10*) naltrexone (0.05 mg/kg); *11*) naloxone (0.05 mg/kg)+ICI 174,864 (0.2 mg/kg); *12*) naltrexone (0.05 mg/kg)+ICI 174,864 (0.2 mg/kg). *$p<0.01$, **$p<0.001$ compared to the control.

Our study showed that selective δ-OR agonists DPDPE, (-)-TAN-67, deltorphin II, as well as selective δ-OR antagonists naltrindole, TIPP[ψ], and ICI-174,864 produce a negative chronotropic effect both *in vitro* and *in vivo*. Therefore, the negative chronotropic effect of these δ-OR ligands is mediated via direct effect on the heart, which is also confirmed by changes in cardiac contractility under the action of δ-OR ligands. It was established that both δ-agonists and δ-antagonists produce a negative inotropic effect on isolated and perfused rat heart.

The nonspecific interaction of δ-OR ligands with ionic channels in cardiomyocyte sarcolemma can be excluded. The examined pharmacological agents had different chemical structure (the only common feature is their high affinity to δ-OR), so it is unlikely that they can exhibit high affinity to the same type of ionic channels. The negative inotropic and chronotropic effects of these ligands were observed at nanomolar concentrations not surpassing 220 nM, which indicates specificity of their effects. It can be hypothesized that the examined effects of δ-OR ligands can result from occupancy of cardiac receptors. Probably, the negative inotropic effect of δ-antagonists can result from their interaction with δ-OR or some other unknown receptor of high affinity to these drugs.

The hypothesis on the involvement of δ-receptors in the discussed cardiotropic effects is favored by the facts that all examined drugs are selective ligands of OR, while cardiomyocytes are characterized by enhanced density of δ-OR [15]. Generally, the interaction of the ligands with the receptor results in activation, inhibition, or activation-inhibition (in this case, the ligands are referred as partial agonists). We established that neutral nonselective OR antagonist naloxone produces no effect on HR and pumping action of the heart (Table 1), so it can be hypothesized that the negative inotropic and chronotropic effects of δ-agonists and δ-antagonists are related to activation of these receptors instead of their inactivation. Therefore, the examined δ-antagonists belong to partial agonists of δ-OR, which can activate and inhibit them.

The data obtained cannot exclude the possibility that δ-ligands interact with so-called non-opioid receptors for opioid peptides [5,9]. The existence of non-opioid receptors for dynorphin in cardiomyocytes is demonstrated [9]. Our data also attest to the existence of these receptors in the heart [1]. We established that opioid peptide dalargin produces negative inotropic and chronotropic effects on isolated heart [1]. Similar effects were observed with des-Tyr-dalargin, a dalargin analog not interacting with OR [1]. However, the non-opioid receptors for opioid peptides exhibit high affinity only towards opioid peptides and practically do not interact with the non-peptide ligands to OR [5,9]. The data obtained here with both peptide δ-OR (TIPP[ψ] and ICI 174,864) and non-peptide δ-OR antagonist naltrindole favor more correct suggestion that δ-antagonists are actually partial OR-agonists. In this case, their effect should not be observed under conditions of δ-receptor blockade with neutral antagonists naloxone or naltrexone. When applied in a dose of 0.05 mg/kg, these antagonists produced no effect on HR (Fig. 1). Affinity of naloxone [13] and naltrexone [10] to δ-OR 6- and 14-times surpassed that of ICI 174,864, respectively. Naloxone, naltrexone, and ICI 174,864 are competitive antagonists of δ-OR [10,13]. Thus, naloxone and naltrexone (0.05 mg/kg) should prevent the interaction of ICI 174,864 with δ-receptors, when the dose of ICI 174,864 is 0.2 mg/kg. However, the negative chronotropic effect of ICI 174,864 persisted when δ-OR were blocked with naloxone or naltrexone in a dose of 0.05 mg/kg (Fig. 1). Therefore, the negative chronotropic effect of ICI

TABLE 1. Effect of OR Ligands on Contractility of Isolated Rat Heart (*M±m*, percents of initial values)

Experimental series	HR	LVPD	MCR	MRR
Control	100±5	100±5	100±8	100±9
DPDPE, 154 nmol/liter	70±9*	49±7*	41±8*	45±6*
(-)-TAN-67, 197 nmol/liter	71±5*	52±7*	58±9*	54±7*
ICI-174,864, 127 nmol/liter	63±11*	57±10*	54±12*	44±10*
TIPP[ψ], 155 nmol/liter	46±10*	44±8*	38±9*	33±7*
Naltrindole, 220 nmol/liter	44±5*	51±6*	57±5*	33±8*
Naloxone, 275 nmol/liter	85±12	95±13	93±6	80±11

Note. MCR, maximum contraction rate; MRR, maximum relaxation rate. *$p<0.05$ compared to the control (initial values). All preparations were applied in a final dose of 0.1 mg/kg.

174,864 results from interaction of this agent with an unknown receptor. Probably, the drop in HR caused by naltrindole or TIPP[ψ] also results from their interaction with this receptor. This hypothesis seems to be appropriate, since there are numerous data demonstrating the fact that pharmacological agents assumed to be the selective ligands to one receptor, interact actually with more than one site. For example, lofentanil was known as a selective agonist of μ-OR [8]. However, the fourth type of OR (ORL1) was discovered [4], which interacted with lofentanil [6]. These new receptors are characterized by low affinity to naloxone and naltrexone, so all the effects of nociceptin (an agonist to ORL1 receptors) persisted after blockade of other OR with naloxone and naltrexone [4]. Although naltrindole, ICI 174,864, and TIPP[ψ] could interact with ORL1 receptors, such a possibility seems to be unlikely, because the molecular structure of ORL1 is similar to that of κ-OR in contrast to δ-OR. Probably, δ-antagonists interact with unknown receptor, which is similar to δ-OR, but cannot be inhibited by naloxone.

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