δ-Opioid Receptor Antagonists Exhibit Properties of Partial δ-Receptor Agonists in Isolated Perfused Heart

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Perfusion of the isolated intact rat heart with Krebs—Henseleit solution containing agonists ((-)-TAN-67, DPDPE, and dalargin) or antagonists of δ-opioid receptors (naltrindole, TIPP[ψ], and ICI 174,864) in a final concentration of 0.1 mg/liter was followed by a decrease in the heart rate, end-diastolic pressure, contraction rate, relaxation rate, and left ventricular developed pressure. Perfusion with a solution containing the δ-opioid receptor agonist DPDPE or δ-antagonists naltrindole, TIPP[ψ], and ICI 174,864 before modeling of global ischemia increased the severity of reperfusion-induced contractile dysfunction in the myocardium. Our results suggest that δ-opioid receptor antagonists *in vitro* exhibit properties of partial δ-receptor agonists.

Key Words: *opioid receptor ligands; isolated perfused heart*

A correct pharmacological studies requires suggests the use of highly selective receptor agonists and antagonists. Various selective agonists and antagonists of μ-, δ-, and κ-opioid receptors (OR) were synthesized over the last 20 years [5,6,9]. Selective antagonists of δ-OR (naltrindole, TIPP[ψ], and ICI 174,864) are believed to act as "pure" antagonists of receptors not exhibiting properties of OR agonists.

Here we studied properties of δ-OR antagonists in the isolated perfused rat heart.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 300-350 g. After thoracotomy the hearts were rapidly removed and placed in cold Krebs—Henseleit solution (4°C). After cessation of spontaneous contractions a cannula was inserted into the ascending aortic arch. Isotonic solution was delivered through this cannula. Open-circuit retrograde perfusion of the heart with Krebs—Henseleit solution was performed by the method of Langendorff. Contractility of the isovolumic perfused heart was measured at a constant pressure of 52 mm Hg. Cardiac contractile activity was recorded using an electromanometer connected to a latex balloon introduced into the left ventricle.

The heart was adapted to normoxic perfusion for 35 min. Global myocardial ischemia was modeled by termination of perfusate supply for 45 min. Observations were continued during reperfusion for 30 min. The experiments were performed with the isolated hearts whose initial contractility corresponded to the standard value estimated in our laboratory. The hearts from intact animals exposed to global ischemia and reperfusion served as the control.

We recorded heart rate (HR, bpm), left ventricular developed pressure (LVDP, mmHg), end-diastolic pressure (EDP, % of the initial value), and maximum rates of contraction and relaxation (mm Hg/sec).

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LVDP was calculated as the difference between systolic and diastolic pressures.

We used the nonselective μ- and δ-OR agonist dalargin (H-Tyr-D-Ala-Gly-Phe-Leu-Arg) [1], its analogue not containing tyrosine (des-Tyr-dalargin, H-D-Ala-Gly-Phe-Leu-Arg), selective agonists of δ_1-OR DPDPE (H-Tyr-D-Pen-Gly-Phe-D-Pen-OH) [6,9] and (-)-TAN-67 ((-)-2-methyl-4aa-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12aα-octahydroquinolino [2,3,3-g] isoquinoline dihydrobromide) [6,9], selective antagonist of μ-OR CTAP (NH_2-D-Phe-Cys-Tyr-D-Trp-Arg-Thr-L-Pen-Thr-NH_2) [6,9], selective antagonists of δ-OR TIPP[ψ] (H-Tyr-Ticψ[CH_2NH]Phe-Phe-OH) [6,9] and ICI 174,864 (N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH) [6,9], and selective antagonist of δ_1-OR naltrindole [6,9].

Dalargin and des-Tyr-dalargin were synthesized at the Laboratory of Peptide Synthesis (Russian Cardiology Research-and-Production Center). Other peptides were synthesized at the Multiple Peptide Systems Company. Naloxone hydrochloride was obtained from Sigma. Naltrindole hydrochloride was obtained from Tocris Cookson Ltd. Krebs—Henseleit solution was prepared using ICN Biomedicals reagents.

The test preparations in a concentration of 0.1 mg/liter were added to the perfusate to evaluate *in vitro* effects. The antagonist of μ-, δ-, and κ-OR naloxone and CTAP were used in concentrations of 300 nmol/liter and 0.19 mg/liter, respectively.

After 20-min adaptation to normoxic perfusion one of the ligands was added to Krebs—Henseleit solution. Normoxic perfusion continued for 10 min. The hearts were washed for 5 min to remove preparations. Global ischemia and reperfusion were modeled. Ligands of OR were dissolved in physiological saline immediately before the experiment and added to the perfusate.

The concentrations of preparations were selected taking into account the results of *in vitro* assessment of inotropic activity of OR ligands on the isolated heart [3,4]. OR ligands were injected intravenously 15 min before isolation of the heart. Experiments were performed with OR agonists (0.1 mg/kg) and antagonists (0.5-2.5 mg/kg) [3,4]. The concentration of intravenously injected free OR ligands not bound to plasma proteins is unknown. In our study the apparent distribution volume was taken as 1 liter/kg. It should be emphasized that the actual amount of body fluid is 0.6 liter/kg. Large hydrophilic molecules (*e.g.*, peptides) can diffuse into the extracellular fluid whose volume does not exceed 0.2 liter/kg [7]. When taken into account that the apparent distribution volume is 0.2 liter/kg, the concentration a peptide injected intravenously in a dose of 0.1 mg/kg and affecting the heart cells corresponds to 0.5 mg/liter. However, the actual concentration of intravenously injected opioid peptides (*e.g.*, enkephalins) in the blood should be much lower since more than 50% enkephalins are bound to to proteins [8]. Peptides are enzymatically hydrolyzed in the plasma. Hence, the actual concentration by a peptide injected in a dose of 0.1 mg/kg and affecting the heart cells is little more than 0.1 mg/liter. Taking into account these data, most ligands of OR were used in a final concentration of 0.1 mg/liter.

The results were analyzed by Student's *t* test.

RESULTS

Normoxic perfusion of the intact myocardium with a solution containing δ_1-OR agonists DPDPE and TAN-67, μ- and δ-OR agonists dalargin and its nonopiate analogue des-Tyr-dalargin, and δ-OR antagonists naltrindole, TIPP[ψ], and ICI 174,864 for 10 min was followed by a decrease in HR (Table 1). The addition of preparations to a final concentration of 0.1 mg/liter to the perfusate decreased LVDP, but increased EDP. These changes were accompanied by a decrease in the maximum rate of contraction and relaxation. However, nonselective OR antagonist naltrexone and μ-OR antagonist CTAP had no effect on HR and parameters of myocardial contraction and relaxation. All compounds producing the negative inotropic and chronotropic effect on the isolated heart act as selective ligands of δ-OR. The only exception is dalargin activating μ- and δ-OR [1] and des-Tyr-dalargin not interacting with OR. *In vitro* experiments showed that D-Ala-Gly-Phe-Leu similar to des-Tyr-dalargin (D-Ala-Gly-Phe-Leu-Arg) cannot activate OR [1].

These data show that agonists and antagonists of δ-OR in a concentration of 0.1 mg/liter have similar effects on pumping function of the heart. It can be hypothesized that selective δ-OR antagonists naltrindole, TIPP[ψ], and ICI 174,864 in a concentration of 0.1 mg/liter exhibit activity of partial δ-OR agonists. Previous studies showed that δ-agonists produce a negative chronotropic effect on the isolated heart and cardiomyocytes [2]. Hence, it is unlikely that they cause blockade of δ-OR. μ-OR are absent on cardiomyocytes and, therefore, cannot be occupied by δ-OR ligands [13]. δ-OR antagonists in specified doses have low affinity for κ-OR and cannot bind to these receptors [6]. There is little likelihood that κ-OR on the sarcolemma of heart cells can be activated. Probably, δ-OR antagonists interact with high-affinity binding sites for des-Tyr-dalargin (*e.g.*, ORL1 receptor or nonopiate dynorphin receptor). These receptors were found on cardiomyocyte membranes [10,11]. The ORL1 receptor is molecularly similar to κ-OR. The International Society of Pharmacologists assigned this receptor to OR [5]. The molecular structure of nonopiate dy-

TABLE 1. Effect of OR Agonists and Antagonists on Contractility of the Isolated Heart (% of Control, M±m)

OR agonists and antagonists	HR	LVDP	EDP	Maximum rate	
				contraction	relaxation
Control	100±5 90.0±6.9	100±5 71.0±5.9^{+}	100±9 251±28^{+}	100±8 68±7^{+}	100.0±8.5 55±8^{+}
DPDPE, mg/liter 0.1	70.0±9.5* 63.0±5.9*	49±7* 37.0±5.8*	208±18* 277±39*	41±8* 31±6*	45.0±5.6 18.0±2.4
0.5	95±10 80.0±9.5	63.0±6.8* 36.0±7.2*	213±26* 261±35*	55±12* 34±6*	68±11* 27±4*
TAN-67, 0.1 mg/liter	71.0±5.4* 83.0±6.8	52±7* 57±5*	171±21* 254±24*	58.0±9.2* 51±12*	54.0±6.7* 39.0±5.6*
des-Tyr-dalargin, 0.1 mg/liter	81.0±6.2* 76.0±3.3*	67.0±4.8* 45.0±5.2*	230±31* 334±34*	59±9* 46.0±5.2*	57.0±5.8* 31.0±4.5*
Dalargin, 0.1 mg/liter	80.0±8.2* 90±6	66±5* 63±4*	209±17* 288±23*	58.0±8.7* 56.6±6.0*	57.5±5.0* 45.0±4.4
Naloxone, 0.3 μM	85±12 76.0±6.3*	95±13 78±9	112±10 261±23*	93.0±5.8 70±10*	80±11 65.0±6.4*
ICI, 0.1 mg/liter	63.0±11.5* 57.0±9.2*	57±10* 37.0±6.7*	185±20* 290±33*	54±12* 34.0±5.6*	44±10* 18.0±2.4*
TIPP, 0.1 mg/liter	46±10* 50.0±5.2*	44.0±8.3* 29.0±6.7*	266±36* 288±37*	38±9* 25.0±3.1*	33.0±7.3* 12.0±1.7*
Naltrindole, 0.1 mg/liter	44.0±5.1* 59±12*	51±6* 30.0±4.8*	210±20* 237±23*	57.0±5.2* 27.0±4.9*	33.0±8.1* 29±3*
CTAP, 0.19 mg/liter	88±9 92±13	93±12 78±8	118±7 278±29*	87±13 76±12	83±10 59±9*

Note. Numerator, 10th minute of perfusion; denominator, 30th minute of perfusion. $p<0.05$: *compared to the control; $^{+}$compared to numerator.

norphin receptors remains unknown. Both receptors interact with peptides not containing tyrosine in the initial region of the polypeptide chain [5,10]. Thus, these receptors can serve as a target for des-Tyr-dalargin or peptide antagonists of δ-OR.

Further experiments were performed under conditions of global ischemia (45 min) and reperfusion (30 min). Global ischemia and reperfusion of the heart were followed by a decrease in HR, LVDP, and maximum rates of contraction and relaxation and an increase in EDP (Table 1). Perfusion of the heart with a solution containing DPDPE increased the severity of pump dysfunction produced by ischemia and reperfusion. The only exception was EDP, which did not differ from the baseline level. Preliminary perfusion of the heart with a solution containing TAN-67, dalargin, naloxone, and CTAP had no effect on pump function of the myocardium during reperfusion. The addition of selective δ-OR antagonists naltrindole, TIPP[ψ], and ICI 174,864)to the perfusate before ischemia produced a decrease in HR, LVDP, and maximum rates of contraction and relaxation (similarly to the δ-OR agonist DPDPE). The effect of des-Tyr-dalargin on the isolated heart during reperfusion was similar to that of DPDPE and δ-OR antagonists. As differentiated from other pharmacological preparations, des-Tyr-dalargin increased EDP.

Our results show that antagonists (naltrindole, TIPP[ψ], and ICI 174,864) and agonist of δ-OR (DPDPE) in a concentration of 0.1 mg/liter have similar effects on pump function of the heart. These data indicate that δ-OR antagonists exhibit properties of δ-OR agonists in the isolated perfused rat heart. It seems unlikely that δ-OR antagonists interact with nonopiate receptors. As distinct from OR ligands, des-Tyr-dalargin increases the degree of reperfusion contracture in the isolated heart. Probably, the myocardium contains high-affinity binding sites for des-Tyr-dalargin. Selective anta-

gonists of δ-OR TAN-6 and DPDPE have the same affinity for δ-OR, selectivity, and molecular weight [6,9,12]. It remains unclear, why these ligands produce different effects on the myocardium.

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