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# Stimulation of μ- and δ-Opiate Receptors and Tolerance of Isolated Heart to Oxidative Stress: the Role of NO-Synthase

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**Abstract**—Preliminary intravenous injection of δ-opiate receptor (OR) agonists DSLET (0.1 mg/kg) or DTLET (0.1 mg/kg) increased tolerance of isolated perfused myocardium to damage by oxidative stress simulated *in vivo* with $FeSO_4$ + ascorbic acid. This manifested itself by a decreased level of creatine phosphokinase (CPK) in the perfusate flowing out of the heart during the oxidative exposure. The preliminary systemic injection of μ-agonists DAMGO (0.1 mg/kg) or DALDA (0.1 mg/kg) failed to affect the release of CPK from the myocardium. The cardioprotective effect of the δ-agonist DSLET was completely abolished by preliminary intravenous injection of the δ-OR antagonist ICI 174,864 (2.5 mg/kg). The intravenous injection of the NO-synthase inhibitor L-NAME (50 mg/kg) also completely abolished the cardioprotective effect of δ-OR stimulation. The preliminary injection of DSLET but not of DAMGO prevented an increase in the level of diene conjugates and a decrease in the activity of superoxide dismutase (SOD) in the isolated myocardium tissue. Thus, the *in vivo* stimulation of δ-OR increased the tolerance of the heart to oxidative stress through activation of NO-synthase and SOD.

*Key words*: opiate peptides, lipid peroxidation, superoxide dismutase, NO-synthase, oxidative stress

Free-radical induced lipid peroxidation (LPO) extremely often occurs in biological membranes and is involved in physiological processes of the cell. Lipid peroxidation contributes somewhat to the pathogenesis of diseases of virtually all systems of the body, including the cardiovascular system [1-4]. Especially important is the role of LPO products and of reactive oxygen species in the pathogenesis of reperfusion-caused damage [5].

Synthetic antioxidants can effectively prevent damage to the myocardium during experimental ischemia and reperfusion but their protective effect is low if they are used immediately before the restoration of coronary perfusion [6], and this significantly limits their therapeutic use. Moreover, many antioxidants display a cardioprotective effect only at very high doses. For example, the protective effect of ionol occurs only at the dose of 50 mg/kg [7]. The use of such high doses of drugs inevitably increases the incidence of undesirable side effects; therefore, the search for new approaches to increase the tolerance of the heart to oxidative stress is an urgent problem of medical biochemistry and pharmacology.

We suggested that activation of opiate receptors (OR) should increase the activity of the endogenous antioxidant system. Thus, we showed earlier that a common agonist of μ- and δ-OR, dalargin, suppressed lipid peroxidation on both the whole organism level [8] and with the isolated perfused rat heart [9]. However, it remained unclear if the activation of μ- and δ-receptors could increase the cardiomyocyte tolerance to oxidative stress. If it could, was this protective effect associated with activation of superoxide dismutase (SOD) and catalase? How can changes in the synthesis of one of the intracellular messengers of NO affect the manifestation of protective effects of opiates? The purpose of the present work was to answer these questions.

## MATERIALS AND METHODS

Experiments were performed on isolated hearts of Wistar rats perfused isovolumically according to Langendorf with oxygenated (95% $O_2$ + 5% $CO_2$, 37 ± 0.5°C) Krebs–Henseleit solution of the following composition (mM): NaCl (120), KCl (4.8), $CaCl_2$ (2.0), $MgSO_4$

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(1.2), $KH_2PO_4$ (1.2), $NaHCO_3$ (20), glucose (10). The isolated hearts were contracting with spontaneous rhythm. LPO was activated after stabilization for 20 min. During the subsequent 20 min of perfusion, the isolated hearts were exposed to oxidative stress by addition into the perfusate of $O_2^{\cdot}$-generating system: $FeSO_4$ (0.2 mmol/liter) + ascorbic acid (0.5 mmol/liter) [9]. The degree of disorder in sarcolemma intactness and, correspondingly, the cardioprotective effect of OR ligands under study was assessed using commercial kits (catalog Nos. 47-50, Sigma, USA) by the activity of creatine phosphokinase (CPK) in the perfusate flowing out of the heart. The result was expressed in μmole NADH/(min·liter). After the experiment, the myocardial specimens were frozen in liquid nitrogen to determine parameters of lipid peroxidation. The content of diene conjugates (DC) was determined by the increase in optical density of hexane extracts of the myocardium at 232 nm [10], and the result was calculated per g tissue. The secondary LPO product malonic dialdehyde (MDA) was determined by reaction with thiobarbituric acid [11]. Activities of antioxidant enzymes superoxide dismutase (SOD, EC 1.15.1.11) and catalase (EC 1.11.1.6) were determined concurrently in the myocardial specimens. The activity of SOD was expressed in mmol/min per mg protein, and it was assessed by the ability of myocardial tissue homogenates of various groups of animals to inhibit the spontaneous oxidation of adrenaline in alkaline medium (pH 10.2) [12]. The purified preparation of superoxide dismutase (catalog No. 190117, ICN Biomedicals, Aurora, Ohio, USA) was used as the standard. The catalase activity of the specimens was determined spectrophotometrically and expressed in mmol/min per g protein [13]. The protein content was determined by the biuret method [14]. The intact state of biochemical parameters was determined for hearts perfused for 40 min with Krebs–Henseleit solution without addition of $FeSO_4$ and ascorbic acid.

The involvement of μ- and δ-OR in the regulation of myocardial tolerance to damage by reactive oxygen species was assessed using selective and common agonists of each type of the receptors. The μ- and δ-OR were activated together by intravenous injection of dalargin (D-Ala$^2$-Leu$^5$-Arg$^6$-enkephalin) [15] at the dose of 0.1 mg/kg 20 min before the heart isolation. To activate μ-OR, the animals were injected with a similar schedule with DAMGO ([D-Ala$^2$,N-Me-Phe$^4$,Gly-ol$^5$]-enkephalin) [16] or DALDA ([D-Arg$^2$,Lys$^4$]-dermorphin-(1-4)-amide) [17] at the dose of 0.1 mg/kg. DSLET ([D-Ser$^2$, Leu$^5$,Thr$^6$]-enkephalin) [18] or DTLET ([D-Thr$^2$, Leu$^5$]enkephalyl-Thr$^6$) [18] were used as selective agonists of δ-OR according to the schedule chosen. The opiate dose was chosen based on earlier published data on the "antioxidant" activity of a nonselective peptide agonist of μ- and δ-OR dalargin [9]. A selective antagonist of δ-OR ICI 174,684 (N,N-dial-Alyl-Tyr-Aib-Aib-Phe-LeuOH, where Aib is α-aminoisobutyric acid) [19] was injected intravenously at the dose of 2.5 mg/kg 30 min before the heart isolation or 15 min before the injection of DSLET. The selective ligands of opiate receptors used in the present work were synthesized by Chiron Mimotopes Peptide Systems (San Diego, USA) and kindly given to us by Dr. K. Gormley (NIDA, USA). Dalargin was synthesized at the Cardiology Research Center, Russian Academy of Medical Sciences.

The lipid-soluble antioxidant dibunol (ionol, Sigma) was used as the preparation for comparison. Dibunol was injected thrice (for two days and 1 h before the experiment) intraperitoneally at the dose of 120 mg/kg [5].

To determine the effect of NO-synthase inhibition on manifestations of cardioprotective and "antioxidant" properties of δ-agonists under conditions of oxidative stress, rats were injected intravenously with an NO-synthase inhibitor L-NAME at the dose of 50 mg/kg [21]. L-NAME was injected either 30 min before the experiment or 15 min before the intravenous injection of DSLET. L-NAME was purchased from ICN Biomedicals (Aurora, Ohio, USA).

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

## RESULTS

The *in vitro* simulation of oxidative stress was accompanied by intensification of LPO in the myocardial tissue that confirmed by 71 and 23% increases in the contents of MDA and DC, respectively (table). The activity of CPK in the perfusate flowing out of the heart was increased 4.5-fold compared to the initial level (figure). Moreover, stimulation of free radical oxidation of lipids significantly (1.4-fold) increased the activity of catalase and 3.5-fold decreased the activity of SOD relatively to similar parameters in the intact animals (table).

Stimulation of μ- and δ-opiate receptors by intravenous injection of the common agonist dalargin before the simulation of oxidative stress promoted a 1.9-fold decrease in the level of CPK in the perfusate flowing out of the heart compared to the control (figure). The use of dalargin limited the accumulation of lipid peroxidation products in the myocardial tissue after the oxidative stress. The contents of MDA and DC in heart homogenates of this group were decreased 34 and 51%, respectively, compared to the control group. However, the activity of SOD was increased twofold compared to the control; the activity of catalase was unchanged.

The figure shows that the preliminary systemic injection of a μ-selective agonist DAMGO failed to influence the release of CPK into the perfusate flowing out of the heart with activated free radical processes. The activation of μ-OR with another peptide, DALDA, also did not increase the tolerance of the myocardium to oxidative stress that was indicated by the high level of CPK in the perfusate from the hearts of this group (figure).

Effect of preliminary injection of opiate receptor ligands and of L-NAME on contents of LPO products and on activities of oxidative enzymes in isolated myocardial tissue subjected to oxidative stress (M ± m)

| Group of animals | MDA, % relative to the intact group | DC, % relative to the intact group | Catalase, % relative to the intact group | SOD, % relative to the intact group |
|---|---|---|---|---|
| Intact, $n = 15$ | 100 ± 5.0 | 100 ± 5.8 | 100 ± 4.9 | 100 ± 3.2 |
| Activation of LPO (control), $n = 15$ | 171.4 ± 11.8<br>$p_1 < 0.001$ | 122.6 ± 3.9<br>$p_1 < 0.01$ | 144.1 ± 9.3<br>$p_1 < 0.001$ | 28.6 ± 3.3<br>$p_1 < 0.001$ |
| Dalargin (0.1 mg/kg, i.v.) + activation of LPO, $n = 12$ | 112.6 ± 5.9<br>$p_1 > 0.05$<br>$p_2 < 0.001$ | 69.9 ± 4.7<br>$p_1 < 0.001$<br>$p_2 < 0.001$ | 129.6 ± 3.5<br>$p_1 < 0.001$<br>$p_2 > 0.05$ | 57.1 ± 3.7<br>$p_1 < 0.05$<br>$p_2 < 0.01$ |
| DAMGO (0.1 mg/kg, i.v.) + activation of LPO, $n = 13$ | 102.5 ± 3.4<br>$p_1 > 0.05$<br>$p_2 < 0.001$ | 120.4 ± 7.9<br>$p_1 < 0.05$<br>$p_2 > 0.05$ | 98.0 ± 4.0<br>$p_1 > 0.05$<br>$p_2 < 0.001$ | 41.8 ± 1.6<br>$p_1 < 0.001$<br>$p_2 < 0.05$ |
| DALDA (0.1 mg/kg, i.v.) + activation of LPO, $n = 13$ | 108.4 ± 5.0<br>$p_1 > 0.05$<br>$p_2 < 0.001$ | 112.4 ± 16.5<br>$p_1 < 0.05$<br>$p_2 > 0.05$ | 134.9 ± 5.1<br>$p_1 < 0.001$<br>$p_2 > 0.05$ | 61.5 ± 4.9<br>$p_1 < 0.001$<br>$p_2 < 0.001$ |
| DSLET (0.1 mg/kg, i.v.) + activation of LPO, $n = 12$ | 114.3 ± 3.4<br>$p_1 > 0.05$<br>$p_2 < 0.001$ | 105.8 ± 3.6<br>$p_1 > 0.05$<br>$p_2 < 0.001$ | 118.2 ± 3.6<br>$p_1 < 0.01$<br>$p_2 < 0.01$ | 100.7 ± 4.6<br>$p_1 > 0.05$<br>$p_2 < 0.001$ |
| DTLET (0.1 mg/kg, i.v.) + activation of LPO, $n = 12$ | 91.6 ± 3.4<br>$p_1 > 0.05$<br>$p_2 < 0.001$ | 102.5 ± 4.9<br>$p_1 > 0.05$<br>$p_2 < 0.01$ | 123.0 ± 2.4<br>$p_1 < 0.001$<br>$p_2 < 0.05$ | 114.5 ± 6.0<br>$p_1 < 0.05$<br>$p_2 < 0.001$ |
| ICI (2.5 mg/kg, i.v.) + activation of LPO, $n = 10$ | 149.6 ± 4.2<br>$p_1 < 0.001$<br>$p_2 > 0.05$ | 129.2 ± 3.3<br>$p_1 < 0.05$<br>$p_2 > 0.05$ | 130.1 ± 5.5<br>$p_1 < 0.001$<br>$p_2 > 0.05$ | 41.5 ± 4.9<br>$p_1 < 0.001$<br>$p_2 > 0.05$ |
| ICI (2.5 mg/kg, i.v.) + DSLET (0.1 mg/kg, i.v.) + activation of LPO, $n = 10$ | 167.2 ± 5.9<br>$p_1 < 0.001$<br>$p_2 > 0.05$ | 131.9 ± 3.6<br>$p_1 < 0.05$<br>$p_2 > 0.05$ | 134.5 ± 4.8<br>$p_1 < 0.001$<br>$p_2 > 0.05$ | 40.3 ± 3.7<br>$p_1 < 0.001$<br>$p_2 > 0.05$ |
| Dibunol (120 mg/kg, i.p.) + activation of LPO, $n = 10$ | 83.2 ± 3.4<br>$p_1 < 0.01$<br>$p_2 < 0.001$ | 49.0 ± 1.9<br>$p_1 < 0.001$<br>$p_2 < 0.001$ | 128.3 ± 3.3<br>$p_1 < 0.001$<br>$p_2 > 0.05$ | 105.1 ± 4.5<br>$p_1 > 0.05$<br>$p_2 < 0.001$ |
| L-NAME (50 mg/kg, i.v.) + activation of LPO, $n = 13$ | 154.6 ± 6.7<br>$p_1 < 0.001$<br>$p_2 > 0.05$ | 122.6 ± 4.7<br>$p_1 < 0.01$<br>$p_2 > 0.05$ | 166.3 ± 4.3<br>$p_1 < 0.001$<br>$p_2 < 0.05$ | 34.8 ± 4.6<br>$p_1 < 0.001$<br>$p_2 > 0.05$ |
| L-NAME (50 mg/kg, i.v.) + DSLET (0.1 mg/kg, i.v.) + activation of LPO, $n = 12$ | 160.5 ± 10.9<br>$p_1 < 0.001$<br>$p_2 > 0.05$ | 139.8 ± 5.5<br>$p_1 < 0.01$<br>$p_2 > 0.05$ | 135.4 ± 2.6<br>$p_1 < 0.001$<br>$p_2 > 0.05$ | 35.2 ± 2.2<br>$p_1 < 0.001$<br>$p_2 > 0.05$ |

Note: $p_1$ is the significance relative to the group of intact hearts; $p_2$ is the significance relative to the group of hearts perfused with $Fe^{2+}$ (0.2 mmol/liter) + ascorbic acid (0.5 mmol/liter) for 20 min.

Nevertheless, the stimulation of μ-OR by DAMGO promoted a 40% decrease in the content of MDA in the myocardium tissue compared to the control group but did not affect the level of DC (table). Similar changes in the levels of LPO products were found in the myocardium specimens subjected to oxidative stress under conditions of DALDA-induced activation of μ-OR. Thus, the level of MDA was 37% decreased, whereas the level of DC was not significantly changed relatively to the control group (table).

Pretreatment of μ-receptors with a selective agonist DAMGO before the *in vitro* simulation of oxidative stress markedly reduced the decrease in activities of antioxidative enzymes that was caused by addition of $FeSO_4$ into the perfusate. But, although the activity of SOD was 1.5-fold increased relative to the stress control group, it remained

two times lower than in the intact specimens of isolated heart (not subjected to oxidative stress), while the capacity of myocardial catalase to split $H_2O_2$ was recovered to normal values (table). Similar changes in SOD were found also after pretreatment with another μ-agonist, DALDA, but the enzymatic splitting of $H_2O_2$ remained at the level of the stress control (table).

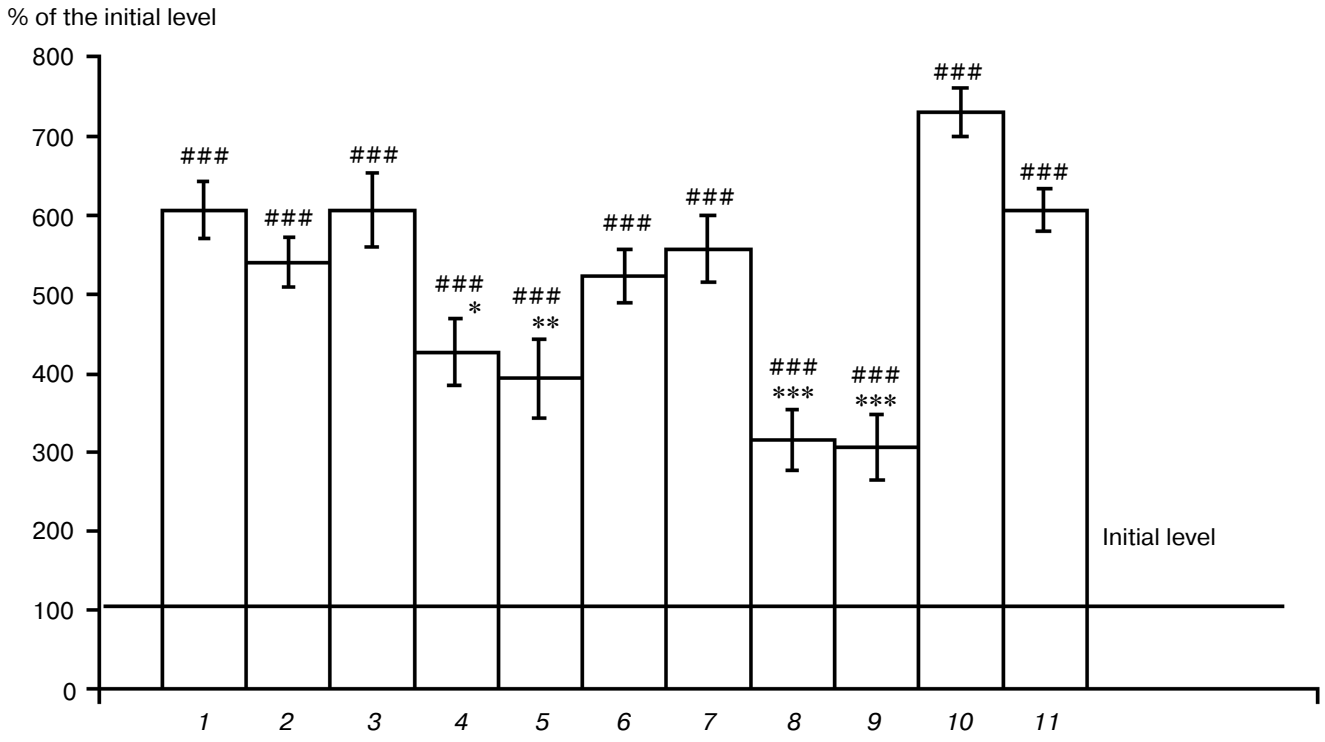
The figure shows that the stimulation of δ-OR by intravenous injection of DSLET or DTLET in both cases was accompanied by a significant 1.4-fold decrease in the level of CPK in the perfusate flowing out of the heart compared to the control group, and this suggested the increased tolerance of cardiomyocytes to the damaging effects of reactive oxygen species.

The cardioprotective effect of the studied δ-agonists was associated with a decrease in the level of LPO products in the myocardium. After the injection of DSLET, the contents of DC and MDA were decreased 14 and 34%, respectively, compared to the hearts subjected to oxidative stress (table). The preliminary systemic injection of DTLET caused a 16% decrease in the level of DC and a 47% decrease in the level of MDA compared to the control values.

Preliminary injections of DSLET or DTLET in our experiments increased the activity of SOD under conditions of oxidative stress up to the level in the intact myocardium. However, the enzymatic splitting of $H_2O_2$ increased on the addition into perfusate of $FeSO_4$ + ascorbic acid was unchanged despite the stimulation of δ-receptors.

One can see from data presented in the table and figure that the selective inhibition of δ-OR by the intravenous injection of ICI 174,684 did not affect the level of CPK in the perfusate, the contents of lipid peroxidation products, and the activities of oxidative enzymes in the myocardium tissue subjected to oxidative stress.

However, the preliminary inhibition of δ-receptors completely eliminated the cardioprotective effect of DSLET that was recorded during the activation of free radical processes. Thus, the CPK level in the perfusate flowing out of the hearts of this group was not significantly different from the control value (figure).




Changes in the activity of creatine phosphokinase in the perfusate flowing out of hearts during activation of free radical processes under conditions of preliminary injection of opiate receptor ligands, the antioxidant dibunol, and L-NAME, an inhibitor of nitric oxide: *1*) group of hearts with activated LPO; *2*) DAMGO + LPO activation; *3*) DALDA + LPO activation; *4*) DSLET + LPO activation; *5*) DTLET + LPO activation; *6*) ICI 174,684 + LPO activation; *7*) ICI 174,684 + DSLET + LPO activation; *8*) dibunol + LPO activation; *9*) dalargin + LPO activation; *10*) L-NAME + LPO activation; *11*) L-NAME + DSLET + LPO activation. Note: significant differences relative to the control group: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Difference relative to the initial values: ### $p < 0.001$. The value of 6.5 μmole NADH/(min·liter) was taken as corresponding to 100% activity of CPK in the perfusate flowing out of the group of intact hearts. The number of animals in the groups was as indicated in the table.

The table shows that the injection of δ-antagonist prevented the δ-receptor-mediated activation of SOD. Moreover, the production of MDA and DC in response to the injection of DSLET was not decreased in this group (table).

A triple intraperitoneal injection of the antioxidant dibunol at the dose of 120 mg/kg resulted in the 1.8-fold decreased CPK level in the perfusate compared to the control group (figure). And this was associated with a decreased intensity of LPO in the myocardial tissue that was manifested by 60 and 52% decreased accumulations of DC and MDA, respectively, relative to the control values (table). The use of dibunol as a protective agent provided a 2.5-fold increase in the activity of SOD compared to the control group. The activity of catalase was not significantly changed (table).

In the next series of experiments, we attempted to assess the contribution of NO to the δ-receptor-mediated cardioprotective and "antioxidant" effects of DSLET under conditions of oxidative stress.

The preliminary injection of the inhibitor of NO-synthase L-NAME eliminated the cardioprotective effect of DSLET under conditions of activated free radical processes. The levels of CPK activity in the perfusate flowing out of the hearts of this group did not differ from those in the control group (figure). The accumulation of LPO products MDA and DC in the myocardial specimens of the experimental group was also not significantly decreased relative to the control (table). The combined use of L-NAME and DSLET was not accompanied by changes in activities of SOD and catalase under conditions of oxidative stress.

The intravenous injection only of L-NAME, an inhibitor of NO-synthase, did not affect the release of CPK from cardiomyocytes subjected to free radical oxidation (figure). Contents of MDA and DC in the myocardial specimens of this group did not differ from those in the control group (table); also, no changes in the activity of SOD were recorded. However, the average value of catalase activity in this group was significantly (70%) higher than in the control heart group (table).

## DISCUSSION

The increased release of CPK, which is a marker of cell injury, into the perfusate suggests that the cardiomyocyte sarcolemma is damaged and that its integrity is disturbed due to exposure to reactive oxygen species generated *in vitro* by the mixture of $FeSO_4$ + ascorbic acid [1].

The membrane integrity during oxidative stress could be disturbed, in particular, due to intensification of LPO, and this was seen by our finding of increased accumulation of MDA and DC in the control myocardial specimens (table). The intensification of free radical reactions can be responsible for damage not only to the external cell membrane but also of membrane structures of cell organelles, including lysosomes, with the associated labilization of lysosomal enzymes, which are mainly proteases and phospholipases [7]. The activation of these enzymes is completed by destruction of cardiomyocytes and the release of CPK into the perfusate, and this is what was found in our experiments.

The simulation of oxidative stress in the isolated myocardial tissue was accompanied by a decrease in the SOD activity and, as a consequence, by accumulation of $O_2^{\cdot}$ and of LPO products. The activity of SOD, in turn, is related to the intensity of free radical lipid peroxidation. Thus, according to some literature data, fatty acid peroxides suppress the activity of SOD [22, 23] through their interaction with the active site of the enzyme [24]. On the other hand, a decrease in SOD activity induced by various factors can by feedback cause an increase in the contents of lipid peroxides.

We suggest that the increased activity of catalase detected under conditions of oxidative stress should be an element of a compensatory reaction in response to the increased level of reactive oxygen species in the cell. The nature of the increased activity of catalase in response to activation of LPO remains unclear.

Analysis of cardioprotective activities of selective agonists of μ- and δ-opiate receptors under conditions of oxidative stress suggests that their effects should be mediated through δ-receptors. This suggestion is also confirmed by results of the combined injection of antagonist and agonist of the same type of receptors (figure). Thus, the preliminary inhibition of δ-OR with ICI 174,684 completely prevented the manifestation of the cardioprotective effect of DSLET during the simulation of oxidative stress, and this directly confirmed the involvement of δ-receptors in the regulation of cardiomyocyte tolerance to the damaging effect of free radicals.

We suggest that the increased tolerance of the myocardium upon the injection of δ-OR agonists should be responsible for their "inhibiting effect" on LPO (table). Our findings show that the injection of δ-OR agonists decreased the accumulation of MDA and DC. This effect of δ-ligands was receptor-mediated. This conclusion is based on the finding that suppression of these receptors before the injection of DSLET was accompanied by significantly increased contents of peroxidation products compared to the group of animals preliminary injected only with DSLET.

Based on our findings, it is suggested that the suppression of LPO should occur due to δ-receptor-mediated stimulation of antioxidative enzymes. The activity of SOD was recovered up to its intact level and the activity of catalase remained higher than normal values in the groups preliminary injected with DSLET or DTLET. It is known that catalase is responsible for inactivation of $H_2O_2$ produced during the SOD-induced dismutation of $O_2^{\cdot}$. In this case, these two enzymes are operating in conjugation, and

a simultaneous increase in their activities under conditions of oxidative stress after the prestimulation of δ-OR provides the utilization of reactive oxygen species and, consequently, promotes the decrease in intensity of free radical oxidation of lipids, and this, finally, provides the cardioprotective effect of stimulation of δ-receptors.

No cardioprotective effect of μ-agonists was found on their systemic injection, and this is most likely because of their low stimulation of SOD (table). It seems that such low μ-receptor activation of SOD was insufficient to adequately protect myocardium against reactive oxygen species. And because of this no complete suppression of lipid peroxidation was found on the stimulation of μ-OR, as was indicated by the retention of the increased level of DC under these conditions (table). The decreased accumulation of MDA in the myocardium of the groups under consideration seems to be the result of suppression of the synthesis of certain prostaglandins because MDA is generated not only during degradation of diene conjugates of fatty acids but also during the production of prostaglandins [25]. Consequently, the decreased production of MDA in response to stimulation of μ-OR was likely to be caused by inhibition of prostanoid biosynthesis. Thus, it was found earlier that DALDA, a selective agonist of μ-OR, suppressed the biosynthesis of thromboxane in myocardium [26].

The combined activation of μ- and δ-opiate receptors by injection of the common agonist of these receptors dalargin in our experiments induced the tolerance of cardiomyocyte sarcolemma to the damaging effect of oxidative stress that manifested itself by decreased release of CPK in the outflowing perfusate. Obviously, the cardioprotective effect of dalargin, similarly to that of δ-agonist, was associated with its capacity to inhibit LPO that was indicated by decreased contents of MDA and DC in the myocardial tissue (table). On the other hand, dalargin failed to induce similar significant changes in the activities of SOD and catalase as in the case of stimulation of δ-receptors by selective ligands. It seems that the inhibition of lipid peroxidation in the heart tissue of this group was caused not only by the increased activities of antioxidative enzymes but also by a modulating effect of dalargin on the synthesis of prostanoids [9], which are involved in lipid peroxidation [27]. Thus, we found that the preliminary injection of dalargin promoted the recovery of synthesis of prostacyclin [9], which has cardioprotective properties [28]. It is suggested that "antioxidant" and cardioprotective effects of dalargin are manifested by the combination of its favorable effects on the synthesis of prostaglandins and on the activities of antioxidative enzymes.

The intraperitoneal injection of dibunol increased the tolerance of myocardium cells to oxidative stress, as confirmed by our observation of the decreased release of CPK into the perfusate flowing out of the hearts of this group. Dibunol is an antioxidant with phenol groups that act as "radical traps"; thus, binding of free radicals by dibunol decreases the intensity of free radical processes, decreases the generation of LPO products, and as a result, protects the active site of SOD against inhibition by peroxides and reactive oxygen species. The increased activity of SOD in response to injection of the antioxidant is clearly associated with this effect.

Data on CPK activity in the animal groups preliminarily injected with DSLET, DTLET, or dibunol suggest that δ-agonists should more efficiently contribute to the tolerance of the myocardium to oxidative stress because their cardioprotective effect comparable to the effect of dibunol could be obtained by injection of lower doses of these peptides. This suggestion is also supported by a significantly shorter period required for development of the "antioxidant" and cardioprotective effects of δ-ligands (DSLET, DTLET) than in the case of dibunol injection.

The question of mechanisms of opiate-related regulation of SOD and catalase activities remains open. Based on the literature on the regulatory effect of NO on SOD activity under conditions of ischemia-reperfusion [29], it is suggested that a similar regulatory effect should also exist under conditions of oxidative stress. Moreover, there are also reports that catalase can be regulated by NO [30, 31]. It is also found that the nitric oxide molecule itself can inactivate certain free radicals [32, 33].

Our experiments with the preliminary injection of L-NAME into animals showed that the inhibition of NO synthesis failed to affect the intensity of LPO and the activity of SOD under conditions of oxidative stress. The increased activity of catalase in the myocardial specimens of this group relative to the control values was most likely caused by a decreased suppressive effect of NO on catalase [30, 31].

The inhibition of NO-synthase before the activation of δ-OR completely abolished the cardioprotective effect of DSLET during the simulation of oxidative stress. Thus, it is reasonable to suggest that NO should be involved in the protective effect of opiate peptides. A concurrent disappearance of the "antioxidant" effect associated with the stimulation of δ-OR was recorded in the myocardial tissue of this group. Therefore, it is suggested that the effect of DSLET on activities of oxidative enzymes and the intensity of LPO should be mediated through the activation of nitric oxide synthesis by NO-synthase. Our findings are in consistence with data of other authors [34] who have shown an increase in the synthesis and activity of Cu,Zn-SOD in the cell culture incubated in a medium containing the NO donor SNAP (S-nitroso-N-acetylpenicillamine). Taking into consideration the ability of nitric oxide to enter the neutralization reaction with free radicals, it seems likely that an increase in the synthesis of NO in response to stimulation of δ-OR should decrease the intensity of free radical processes. In this case, changes in activities of SOD and catalase should be only a consequence of decreased contents of peroxides and free radicals, which could inhibit SOD [24] as mentioned above.

However, the question remains open why agonists of μ-OR failed to display protective properties under conditions of oxidative stress, while a positive correlation was found between endomorphine-induced activation of μ-opiate receptors and an increase in the synthesis of NO [21].

Thus, our findings indicate that the *in vivo* activation of δ-receptors contributes to the increase in *in vitro* myocardial tolerance to oxidative stress. Decreased contents of lipid peroxidation products and increased activity of superoxide dismutase were concurrently found in myocardium. These protective effects are mediated through δ-receptors and depend on the intensity of NO synthesis in the myocardium. The activation of μ-receptors did not affect the myocardial tolerance to oxidative stress.

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