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Opioids as Triggers of the Adaptive Phenomenon of Ischemic Preconditioning of the Heart

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Transient ischemia/reperfusion has been shown to evoke increases in enkephalin levels in myocardial tissue. Blockade of δ opioid receptors eliminates the cardioprotective effect of ischemic preconditioning both in vivo and in vitro. Inhibition of $\varkappa_1$ opioid receptors eliminates the cardioprotective effect of preconditioning only in vitro. Agonists of μ, δ_1, δ_2, and $\varkappa_1$ opioid receptors imitate the cardioprotective effect of preconditioning. These results indicate that endogenous opioid peptides are triggers of ischemic preconditioning.

Keywords: heart, opioids, ischemic preconditioning.

The adaptive phenomenon of ischemic preconditioning (IP) was discovered in 1986 by Murry et al., [55]. This was almost 30 years ago and interest in studies of this phenomenon has continued. For an endogenous biologically active substance to be regarded as a trigger for IP, it must fulfil the following criteria: its level in the myocardium must increase in response to transient ischemia/reperfusion; blockade of the receptors for this substance must eliminate the cardioprotective effect of IP; exogenous analogs of the substance must imitate the infarct-limiting effect of preconditioning. We believe that opioids match these criteria perfectly.

The Role of Endogenous Opioids in Preconditioning. We have previously demonstrated that after 3 min of exposure of isolated hearts to global ischemia, met-enkephalin levels in the myocardium increased two-fold and leu-enkephalin levels increased 1.5-fold. [7]. Increasing the duration of ischemia to 10 min did not produce any further increase in enkephalin levels. Thus, opioid peptides may aspire to the role of triggers for IP, as their levels in the myocardium increase in response to transient ischemia.

The Role of Opioid Receptors in Preconditioning. In 1995, studies directed by Professor G. J. Gross using experiments performed on rats showed that the nonselective opioid receptor antagonist naloxone eliminated the infarct-limiting effect of IP [63]. Experiments on rabbits established that the cardioprotective effect of preconditioning was eliminated by (–)-naloxone but not by (+)-naloxone, which has low affinity for opioid receptors [24]. Naloxone eliminated not only the cardioprotective, but also the antiarrhythmic effect of IP [43]. Studies on rats yielded data showing that naloxone methiodide, which does not cross the blood-brain barrier (BBB), prevented expression of the infarct-limiting effect of IP [65]. Thus, the cardioprotective effect of IP is due to activation of peripheral opioid receptors. Four types of opioid receptor are known: μ, δ, ϰ, and ORL1 (opioid receptor-like), which are divided into subtypes [10, 25]. Experiments on rats have shown that the selective δ_1 antagonist 7-benzylidenenaltrexone (BNTX) completely eliminated the infarct-limiting effect of IP, while the μ antagonist β-funaltrexamine, the δ_2 antagonist naltriben, and the ϰ antagonist nor-binaltorphimine had no effect on the cardioprotective action of preconditioning [66]. In one study published in 1998 [72], the infarct-limiting effect of IP was assessed in terms of the ratio of the infarct zone to the risk zone (IZ/RZ), where

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the risk zone was defined as that part of the myocardium subject to the actions of ischemia and reperfusion. The authors established that in controls, the IZ/RZ ratio after 30-min coronary occlusion and subsequent reperfusion was 68%, compared with 13% in preconditioned individuals. When animals received naloxone injections before IP, this ratio increased to 27%, while administration of the δ antagonist naltrindole gave IZ/RZ = 25%. The selective κ antagonist nor-binaltorphimine had no effect on infarct size. In addition, these researchers showed that naloxone had no effect on the cardioprotective effect of IP using 20- and 40-min coronary occlusion and reperfusion. The experimenters concluded that there is a "time window" during which endogenous opioids have an infarct-limiting effect. This "window" is covered by 30-min ischemia [72]. A group of American physiologists subjected isolated rabbit hearts to 30-min coronary occlusion and 2-h reperfusion [54]. Preconditioning was produced using a single session of ischemia (5 min) and reperfusion (10 min) or three sessions of ischemia/reperfusion. Naloxone eliminated the infarct-limiting effect of single sessions of preconditioning but had no effect on the cardioprotective effect of three-session IP. Studies reported in 2001 involving experiments on isolated rat hearts showed that naloxone eliminated the positive inotropic effect of IP during myocardial reperfusion [37].

These data provide evidence that the infarct-limiting effect of IP is due to activation of cardiac δ_1 OR. Furthermore, these data indicate that the cardioprotective effect of IP may also involve other triggers, such as bradykinin and adenosine [12, 18, 28].

In 1999, Tomai et al. [71] published the results of their observations of patients who had undergone coronary angioplasty. In essence, this procedure involves inflation and deflation of an intracoronary balloon at the site of an atherosclerotic plaque. During angioplasty, the myocardium is subject to transient ischemia/reperfusion, which is analogous to ischemic preconditioning. Myocardial ischemia was evaluated in terms of ST segment displacement. During the second session of inflation/deflation, ST segment displacement was smaller than after the first session of ischemia/reperfusion. Injection of naloxone eliminated the antiischemic effect of IP. These facts led the authors to conclude that endogenous opioids are involved in cardiac preconditioning in humans [71].

In 2001, Wang et al. [75] reported the results of their experiments on isolated perfused rat hearts subjected to 30-min regional ischemia and 2-h reperfusion. Ischemic preconditioning was produced using two sessions of regional ischemia (5 min) and reperfusion (5 min). The selective κ antagonist nor-binaltorphimine and the δ antagonist naltrindole eliminated the infarct-limiting effect of IP. Nor-binaltorphimine eliminated the antiarrhythmic effect of IP, while prior use of naltrindole had no effect on the IP-induced increase in the resistance of the heart to the arrhythmogenic action of ischemia/reperfusion [75]. The selective κ OR agonist U50,488 had infarct-limiting and antiarrhythmic effects. The δ OR agonist D-Ala^2, D-Leu^5-enkephalin (DADLE) showed only cardioprotective properties. The authors concluded that stimulation of κ OR by endogenous opioids provides the infarct-limiting and antiarrhythmic effects of IP, while δ OR are involved in mediating only the cardioprotective effect of preconditioning [75]. These data are significantly different from results reported by other investigators, who demonstrated that κ OR do not have a role in preconditioning [66, 72]. The cause of these differences is unknown, though attention should be drawn to the fact that the study of Wang et al. [75] was performed on isolated perfused hearts, while the other two studies were performed in vivo.

Thus, it currently appears that δ_1 opioid receptors have a role in ischemic preconditioning. The question of the involvement of κ OR in the cardioprotective effect of preconditioning continues to be discussed. The role of ORL1 receptors in preconditioning remains unstudied.

Exogenous Opioid Receptor Agonists Imitate the Adaptive Phenomenon of Ischemic Preconditioning. The ability of morphine to imitate the phenomenon of preconditioning was first demonstrated in 1996 by Schultz et al. [64]. These investigators established that i.v. injection of morphine to rats at a dose of 0.3 mg/kg before coronary artery occlusion and reperfusion decreased the IZ/RZ ration by a factor of 4.5. A year later, these same authors [65] observed that the infarct-limiting effect of morphine was linked with activation of δ opioid receptors, for which morphine has moderate affinity. Studies on the cardioprotective properties of morphine in rabbits were reported in 1998 [54]. Drug was given i.v. at doses of 0.3, 0.8, and 3 mg/kg. Only the dose of 3 mg/kg was found to have an infarct-limiting effect in rabbits [54]. It remains unclear why a decrease in the IZ/RZ ratio in rats can be achieved with a dose of 0.3 mg/kg, while limiting infarct size in rabbits requires a 10-fold greater dose. Studies in 1999 showed that morphine increases the resistance of isolated cardiomyocytes to hypoxia lasting 90 min [47]. A Chinese physiology group reported data in 2004 supporting results obtained by the American group on the cardioprotective activity of morphine. Morphine was given i.v. at a dose of 8 mg/kg. There is no answer in their report to the question of why they worked with such a high opioid dose. The infarct-limiting effect of morphine was found not to be apparent in conditions of blockade of μ OR, δ OR, or κ OR [80]. Thus, all three receptor types have roles in producing the cardioprotective effect of morphine. Physiological studies yielded further confirmation of the infarct-limiting effect of morphine (0.3 mg/kg, i.v.) [48].

Experiments on isolated perfused hearts reported in 2007 showed that tramadol (an OR agonist-antagonist) also increases the resistance of the heart to ischemia/reperfusion [20]. Results from a clinical trial of tramadol were published in 2010 [74]. Patients received tramadol at a dose of 200 mg at 19:00 before aorto-coronary shunting (ACS), with a repeat dose 6 h before surgery. Surgical intervention

was accompanied by global ischemia/reperfusion of the heart. Tramadol was found to promote increases in blood troponin I levels (a marker of cardiomyocyte necrosis) in the patients. This is evidence that this opioid enhances ischemic and reperfusion damage to the heart in conditions of ACS. These data contradict results from experiments reported by Bilir et al. [20], which provided evidence supporting a cardioprotective effect of tramadol. This fact convincingly shows that the results of any experimental study require clinical verification.

A Korean pharmacology group observed in 2010 that the ultra-short-acting OR agonist remifentanil has a cardioprotective effect in vitro [40]. Unfortunately, the authors of this publication did not address the receptor aspect of the infarct-limiting effect of remifentanil. A clinical trial of remifentanil was performed in China in 2010 [78]. The study included patients undergoing ACS under general anesthesia (propofol + fentanyl). Some of the patients ($n = 20$) received remifantanil (1 μg/kg, i.v. followed by infusion at 0.5 μg/kg for 30 min). During the 24 h following surgery, the myocardial necrosis markers creatine phosphokinase-MB (CPK-MB) and troponin I were measured in plasma. CPK-MB and troponin I levels were found to be significantly lower in patients given remifentanil [78]. Thus, remifentanil has a cardioprotective effect not only in experimental animals, but also in patients with global ischemia/reperfusion of the heart.

Data reported by Takasaki et al. [70] indicate that addition of the μ- and δ OR agonist met-enkephalin to the medium in which cardiomyocytes were being incubated increased cell tolerance to the action of hypoxia-reoxygenation. These same authors subsequently showed that the cytoprotective effect of met-enkephalin was associated with activation of δ OR, as it was not detectable when δ OR were blocked with naltrindole [21]. Studies in 2003 established that i.v. infusion of rabbits with met-enkephalin for 24 h using an osmotic minipump produced a 60% decrease in the IZ/RZ ratio [44]. However, experiments on mice given 24-h met-enkephalin infusions reported by the same group did not confirm the infarct-limiting effect of this peptide. It is clear that there were species differences in the body's response to this and other opioids. This is supported by the fact that the infarct-limiting effect of morphine in rats was detected at a dose of 0.3 mg/kg [65], while a dose of 3 mg/kg was required to produce the same effect in rabbits [54].

Schultz et al. [67] published a report in 1998 addressing the cardioprotective properties of the selective δ_1 agonist TAN-67 (10 mg/kg, i.v.). A year later, Kevelaitis et al. [39] reported experiments on isolated perfused hearts and found that the peptide δ agonist DADLE had a cardioprotective effect in conditions modeling global myocardial ischemia/reperfusion. Subsequent experiments on isolated hearts supported the infarct-limiting effect of DADLE [19]. These investigators established that this effect of DADLE was not apparent after blockade of δ-opioid receptors with naltrindole. A South African group of physiologists [73] observed that i.v. DADLE (10 mg/kg) given before coronary occlusion and reperfusion in vivo produced a three-fold decrease in the IZ/RZ ratio. This effect of DADLE was not seen after injection of naltrindole. This indicates that the infarct-limiting effect of DADLE is linked with activation of δ OR. The cardioprotective properties of DADLE were confirmed in later experiments [36, 68]. In vivo studies showed that this peptide had infarct-limiting effects in rats at a dose of 1 mg/kg given i.v. [36]. However, experiments in pigs, reported by Sigg et al. [69], did not identify the infarct-limiting effect of DADLE (1 mg/kg, i.v.). In 2009, Gross et al. [31] found that the nonselective OR agonist methadone (0.3 mg/kg i.v.) had infarct-limiting properties due to activation of δ OR.

The ability of the δ_1 agonist TAN-67 to imitate the antinecrotic effect of preconditioning was confirmed in later studies both in vivo [27] and in vitro [11, 17, 35]. In 2001, McPherson and Yao [52] observed that the δ agonist BW373U86 increased the resistance of isolated cardiomyocytes to the action of hypoxia and reoxygenation. The cardioprotective properties of TAN-67 and BW373U86 were confirmed in in vivo experiments in a model of coronary occlusion and reperfusion [30, 57, 58]. One of these studies [58] established that the infarct-limiting effect of BW373U86 was due to activation of δ_1 OR. In 2003, Fischbach et al. reported experiments on isolated perfused hearts [26] which showed that addition of the δ agonist SNC-80 to the perfusate before 30-min coronary occlusion and 2-h reperfusion produced an almost three-fold decrease in the IZ/RZ ratio. In 2002, experiments on pigs reported by Sigg et al. [69] identified an infarct-limiting effect with the selective peptide δ_1 agonist H-Tyr-D-Pen-Glycine-Phe-D-Pen-OH (DPDPE, 1 mg/kg). Experiments on rats using 45-min coronary occlusion and 2-h reperfusion performed by the authors did not confirm the data reported by Sigg et al. on the cardioprotective properties of DPDPE [13, 51]. Peptide was given i.v. at doses of 0.1 and 1 mg/kg. Neither dose of the δ_1 agonist affected the IZ/RZ ratio. The cause of this contradiction between our data and the results reported by Sigg et al. lies in the fact that the authors of this study used rats in their experiments, while the American group used pigs. The possibility that there are notable differences between these species in their responses to opioids cannot be excluded. Our experiments on isolated perfused hearts [3, 6] showed that DPDPE at a final concentration of 0.1 mg/liter prevented necrosis of cardiomyocytes induced by ischemia/reperfusion. Our data [8, 17] indicate that the cytoprotective effect of DPDPE was not apparent in conditions of blockade of δ OR by naltrindole. This indicates that the cardioprotective effect of DPDPE resulted from stimulation of δ OR. The infarct-limiting action of DPDPE in experiments on isolated perfused hearts was confirmed by Huang et al. [34]. In 2006, Watson et al. [77] showed that the δ_1- and δ_2 OR agonist ARD-353 (0.3 mg/kg) has an infarct-limiting effect in rats with occlu-

sion of the coronary artery and reperfusion. This protective effect was not apparent in conditions of δ_1 OR blockade with BNTX. Furthermore, Watson et al. [77] established that ARD-353 did not cross the BBB. These facts led to the conclusion that the cardioprotective effect of ARD-353 was due to activation of peripheral δ_1 OR [77]. In 2006, the group led by Professor Gross established [29] that the δ agonist fentanyl isothiocyanate had a marked cardioprotective effect on i.v. administration at a dose of 0.02 mg/kg. Thus, it would appear that δ_1 OR agonists simulate the phenomenon of ischemic preconditioning.

In 2002, Sigg et al. reported experiments on pigs [69] which demonstrated an infarct-limiting effect of the peptide deltorphin D (a presumptive δ_2 agonist) at a dose of 1 mg/kg i.v. In 2009, our experiments on rats with coronary occlusion (45 min) and reperfusion (2 h) [50] confirmed the data reported by Sigg et al. [69]. The selective δ_2 agonist deltorphin II at a dose of 0.12 mg/kg was found to be able to decrease the IZ/RZ ratio. The infarct-limiting effect of deltorphin II persisted after injection of the δ_1 antagonist BNTX but was not apparent after blockade of δ_2 receptors with naltriben [50]. We were unable to identify the infarct-limiting effect of deltorphin II after blockade of peripheral OR with naloxone methiodide (5 mg/kg). Thus, stimulation of peripheral δ_2 OR simulates the adaptive phenomenon of preconditioning.

Professor Wong's group reported [79] that the resistance of isolated cardiomyocytes to the action of sodium cyanide could be increased by adding the selective κ_1 agonist U50,488 to the cell incubation medium. In 2001, this same group of investigators [75] found in experiments on isolated perfused rat hearts that U50,488 (10 μM) decreased the IZ/RZ ratio. Our experiments on isolated perfused rat hearts showed that U50,488 increased the tolerance of the myocardium to global ischemia/reperfusion [4–6]. Studies reported in 2002 [73] showed that i.v. administration of U50,488 (10 mg/kg) to rats before coronary occlusion and reperfusion decreased the IZ/RZ index by a factor of two. The cardioprotective effect of U50,488 was not apparent after injection of the κ antagonist nor-binaltorphimine. Thus, it appears that the protective effect of U50,488 is linked with activation of κ OR.

An American group found [21] that addition of an endogenous peptide agonist of κ OR to the incubation medium for isolated heart cells increased the resistance of cardiomyocytes to the effects of 3-h hypoxia. This effect was not identified in conditions of blockade of κ OR with GNT1. In 2004, Peart et al. [58] reported in vivo experiments in which the κ OR agonists U50,488 and ICI 294,448 had infarct-limiting effects. Preliminary blockade of κ OR with nor-binaltorphimine eliminated this effect. The infarct-limiting effect of the κ OR agonist BRL 52537 persisted in conditions of inhibition of κ OR [58]. Thus, the cardioprotective effects of U50,488 and ICI 204,448 resulted from activation of κ receptors, while the protective effect of BRL 52537 are independent of κ OR activation. As ICI 204,448 does not cross the BBB [62], there are grounds for suggesting that activation of peripheral κ OR promotes increases in the tolerance of the heart to the actions of ischemia/reperfusion. As already noted, the cardioprotective effect of U50,488 was observed in experiments on isolated hearts. It would thus appear that κ OR regulating the resistance of the heart to the actions of ischemia/reperfusion are located in the myocardium. Our experiments on rats with coronary occlusion (45 min) and reperfusion (2 h) were designed to reproduce experiments reported by American investigators [13, 51]. The selective κ_1 OR agonist (±)-U50,488 (0.1 mg/kg or 0.7 mg/kg i.v.) had no effect on the IZ/RZ ratio. We did not see any infarct-limiting effect with the selective κ_2 agonist GR-89696 (0.08 mg/kg) [51]. It is possible that the contradiction between our data and the results reported by American scientists [58] come about because we used (±)-U50,488 racemate while the American group used the more selective agonist (–)-U50,488.

Our experiments reported in 2000 on isolated perfused hearts showed [1] that addition of the selective μ OR agonist H-Tyr-D-Ala^2-Gly-N-Me-Phe-Gly^5-ol (DAMGO) to the perfusion solution prevented necrosis of cardiomyocytes induced by global ischemia and reperfusion. This effect of DAMGO was confirmed by our later experiments [1, 9]. These established that the selective peptide μ agonist NH_2-Tyr-D-Arg-Phe-Lys-NH_2 (DALDA) was also able to prevent myocardial cell death during global ischemia and reperfusion of isolated hearts. We found that i.v. administration of DAMGO (0.1 mg/kg) 15 min before isolation of hearts also increased the resistance of the myocardium to the action of ischemia/reperfusion in vitro [2, 9]. In 1998, Schultz et al. observed [66] that DAMGO (0.1 mg/kg, i.v.) had no effect on the IZ/RZ ratio in rats with coronary occlusion and reperfusion. Our experiments on rats with coronary artery occlusion (45 min) and reperfusion (2 h) addressed the cardioprotective properties of the selective μ OR agonists dermorphin H (0.12 mg/kg) and DAMGO (0.08 or 0.8 mg/kg) [13, 51]. These experiments found that neither of these peptides affected the IZ/RZ ratio. It remains unclear why DAMGO has cardioprotective effects in vitro but has no effect on the IZ/RZ ratio in vivo.

It is not long since we published our in vivo experiments on rats with coronary occlusion (45 min) and reperfusion (2 h) [13, 51], where we sought to evaluate the role of ORL1 receptors. The selective ORL1 receptor agonist was the endogenous peptide nociceptin. Our studies used this peptide at doses of 0.4 and 2.2 mg/kg. The ORL1 receptor agonist had no effect on the IZ/RZ ratio at either of the doses tested [13, 51]. We believe that it is too early to come to any final conclusion that ORL1 receptors are not involved in controlling the resistance of the heart to the action of ischemia/reperfusion, as experiments on the effects of nociceptin on isolated hearts have not been performed. We have already presented data showing that peptide μ agonists have

cardioprotective effects in in vitro experiments but have no effect on the IZ/RZ ratio in vivo. It may be that nociceptin acts in the same way.

The relationships cited above indicate that activation of opioid reports increases the tolerance of the heart to the actions of ischemia/reperfusion, though there is a series of reports providing evidence that stimulation of opioid receptors may worsen ischemic and reperfusional heart damage. Thus, studies in 1979 showed that intravenous administration of morphine to patients at a dose of 2.1 mg/kg depressed the ST segment, this being a sign of myocardial ischemia [42]. Studies reported by Kisin et al. in 1979 [41] showed that i.v. morphine (1 mg/kg) enhanced elevation of the ST segment in cats with coronary occlusion, which was evaluated as an increase in cardiac ischemia. It should be noted that they did not measure the IZ/RZ ratio, leaving room for doubt as to whether morphine increased ischemic heart damage. However, no-one has evaluated the effect of morphine at a dose of 1 mg/kg on the IZ/RZ ratio in cats, so the possibility that ischemic damage to the myocardium is increased in cats after administration of this dose of morphine cannot be completely excluded. In 1982, the same investigators [49] obtained data showing that morphine (3 mg/kg) can increase infarct size in rats. Opiate was given i.v. 10 min before 48-h coronary occlusion without reperfusion. The authors determined infarct size but did not evaluate the IZ/RZ ratio, which would now be regarded as an methodological error. Data reported by Markiewicz et al. [49] contradicted the results of a Chinese physiology group who showed that morphine at a dose of 8 mg/kg decreased the IZ/RZ ratio [80]. These factors cast doubt on the significance of the data reported by Markiewicz et al. Lee and Wong reported in 1985 [46] that addition of naloxone to concentrations of 1.1 or 3.6 mM to the perfusion solution decreased ischemic and reperfusion damage in isolated hearts. It should be noted that the IC_{50} values (the concentration giving half-maximal inhibition) of naloxone in relation to μ OR and δ OR were 8.2 nM [45], while Roques et al. [60] obtained a K_i (inhibition constant) for naloxone against μ OR of 3.4 nM, compared with 50 nM against δ OR. This information is evidence that the cardioprotective effect of naloxone is evidently due to the nonspecific membrane-stabilizing effect of this antagonist and is not a result of OR blockade. It should be noted that most of our experiments did not demonstrate cardioprotective properties for naloxone or other OR antagonists. Almost all other authors have also found no cardioprotective effects with OR blockers. The exception is a report by Chen et al. [23]. Experiments on isolated perfused rat hearts modeled global ischemia/reperfusion and OR antagonists were added to the perfusate at the start of reperfusion. Perfusion with solution containing OR ligands was continued for 10 min. Cardiomyocyte necrosis was evaluated in terms of the IZ/RZ ratio (the risk area was taken as the whole of the left ventricle) and the CPK-MB level in perfusate flowing from hearts [23]. Antagonists were used at the following concentrations: naloxone 10 nM, naltrindole 5 nM, and nor-binaltorphimine 5 nM. All three OR antagonists decreased the IZ/RZ ratio and CPK-MB release. These antagonist concentrations approach the K_i or IC_{50} values [33, 45, 60], so the infarct-limiting effect of OR antagonists cannot be explained in terms of membrane-stabilizing action. Our in vivo experiments [14] showed that i.v. treatment of rats with the μ OR agonist CTAP (1 mg/kg) before coronary occlusion and subsequent reperfusion promoted decreases in the IZ/RZ ratio. It is possible that the cardioprotective effect of CTAP is linked with activation of somatostatin receptors, for which CTAP has moderate affinity [38]. Somatostatin is known from published data [75] to limit the IZ/RZ ration in in vivo experiments, so our suggestion that the mechanism of the infarct-limiting effect of CTAP is grounded in evidence.

These data provide evidence of the negative role of opioid receptors in regulating cardiac tolerance to the actions of ischemia/reperfusion. Results from our experiments [3, 17] show that there is a pool of opioid receptors or nonopioid receptors whose activation by opioids has negative influences on cardiac tolerance to ischemia/reperfusion. Thus, our experiments on isolated perfused rat hearts [3, 17] showed that the cardioprotective effect of DPDPE disappeared when the opioid concentration in the perfusion solution was increased to 0.5 mg/liter. The group led by Professor Gross reported [59] experiments on isolated perfused mouse hearts subjected to global ischemia/reperfusion. They did not find any cardioprotective effect with morphine, which was used at a final concentration of 10 μM. Experiments reported by Miki et al. [54] on isolated perfused rabbit hearts demonstrated an infarct-limiting effect of morphine at a final concentration of 0.3 μM. It seems very likely that the morphine concentration of 10 μM was so high that morphine acted on a pool of opioid or nonopioid receptors whose activation decreases myocardial tolerance to ischemia/reperfusion. In 2004, experiments on isolated perfused hearts reported by Peart and Gross [59] failed to detect any decrease in lactate dehydrogenase from the myocardium during reperfusion after use of BW373U86, which they used in experiments on isolated perfused mouse hearts at a concentration of 1 μM. According to data from other authors [52], BW373U86 at a final concentration of 10 pM increases the resistance of isolated heart cells to the action of hypoxia and reoxygenation. It is possible that the BW373U86 concentration of 1 μM was also too high for this opioid to stimulate δ OR only. These investigators did not find any cardioprotective effect with U50,488 used at a concentration of 1 μM [59]. It should be noted that the IC_{50} of U50,488 is 7.4 nM [45]. A group of physiologists reported in vitro experiments [54] and recommended using opioids at a concentration 10 times greater than the K_i or IC_{50}. One possible explanation for the lack of a protective effect of opioids in experiments on isolated mouse hearts may be species differences in the responses of the mouse and rat

hearts to OR ligands. Aitchison et al. [19] reported data from their experiments on isolated perfused rat hearts. In these experiments, DADLE (10 nM) decreased the IZ/RZ ratio, while an increase in its concentration to 1 μM markedly weakened the cardioprotective effect. Blockade of κ opioid receptors with nor-binaltorphimine increased the cardioprotective effect of high DADLE concentrations. These results are reminiscent of our own data, discussed above [3, 17]. In addition, Aitchison et al. [19] found that the nonselective κ agonist bremazocine (30 nM) increased the IZ/RZ ratio in experiments on isolated hearts. This negative effect of bremazocine disappeared when κ OR were inhibited. In this regard, it should be noted that U50,488 is a selective κ_1 agonist and bremazocine is a κ_2 agonist [61]. Thus, there are grounds for suggesting that stimulation of κ_2 OR decreases the tolerance of the heart to ischemia and reperfusion.

Thus, the data presented here provide evidence that δ_1, δ_2, and κ_1 OR agonists imitate the preconditioning phenomenon. μ OR agonists imitate the ischemic preconditioning phenomenon only in experiments on isolated perfused hearts. The importance of ORL1 receptors in regulating the resistance of the heart to the actions of ischemia/reperfusion remains insufficiently studied. Receptors (presumptively κ_2 OR) exist whose activation by opioids decreases the resistance of the heart to reperfusion damage. The group with the greatest potential for creating cardioprotective agents consists of the agonists of δ_1 OR, δ_2 OR, and κ_1 OR.

Signal Mechanism of the Cardioprotective Action of Opioids. The current view is that $G_{i/o}$ proteins operate as intermediate components between opioid receptors and protein kinases mediating intracellular signaling. A number of studies have demonstrated that protein kinase C (PKC) has a role in mediating the infarct-limiting and antiapoptotic actions of opioid receptor agonists [16]. Protein kinases PI3 (phosphatidylinositol 3) and Akt (kinase isolated from the AKR thymoma cell line) are also involved in forming the cardioprotective effects of opioids [16]. Protein kinases MEK1/2 (mitogen-activated protein kinase kinase), ERK1/2 (extracellular signal-regulated kinase), Src (sarcoma tyrosine kinase), and JAK2 (Janus kinase) play an important role in the cardioprotective effects of opioids [16]. The question of the involvement of JNK kinase (c-Jun N-terminal kinase), p70s6K kinase (70-kDa ribosomal protein s6 kinase), and GRK-2 kinases (G protein-coupled receptor kinases) in the opioid-induced increase in the tolerance of the heart to the actions of ischemia and reperfusion continues to be studied. NO synthase plays an important role in the mechanism of the infarct-limiting actions of opioids [16]. Reactive oxygen species are intracellular messengers for the cardioprotective effect of opioids. Transactivation of opioid receptors and EGFR (epidermal growth factor receptor) is the linking component between opioid receptors and the ERK1/2 and PI3 kinase cascades, MPT-pores (mitochondrial permeability transition pores), K_{ATP} channels (ATP-sensitive K^+ channels), and mitoBK_{Ca} channels (mitochondrial big conductance Ca^{2+}-dependent K^+ channels) [15, 16, 22]. MPT pores, mitoBK_{Ca} channels, and K_{ATP} channels are the most likely candidates for the role of end effectors in opioid preconditioning. In all probability, cells contain several signal cascades mediating the cardioprotective effect of opioids. One of these is as follows: opioids → Src transactivation → PI3K → PI3 phosphates + PDK → Akt → Bax and Bcl-2 → MPT pores → increased cardiac tolerance to the actions of ischemia/reperfusion [16, 56], where PDK is 3'-phosphoinositide-dependent kinase-1/2 and proteins regulating the permeability of MPT pores, Bcl-2 is B-cell lymphoma protein-2, and Bax is Bcl-2-associated X-protein. Data have been obtained supporting the existence of the following signal chain: μ or δ OR → Src → EGF receptors → Ras → Raf-1 → MED1/2 → ERK1/2 → cytoprotection [16, 22], where Ras is rat sarcoma protein and Raf-1 is rapidly accelerated fibrosarcoma serine/threonine protein kinase. Data supporting another signal cascade have been reported: opioids → OR → $G_{i/o}$ proteins → phospholipase C → DAG → PKC → GSK-3β → closed MPT pores → suppression of apoptosis [16], where DAG is diacylglycerol and GSK-3β is glycogen synthase kinase-3β. The following signal pathway has been suggested: opioids → OR → RTK → PI3K → Akt → eNOS → NO → GC → PKG → PKCε → mitoK_{ATP} channels → ROS + MPT pores. The end effectors of this pathway are MPT pores and mitoK_{ATP} channels [16, 32, 53], where RTK is receptor tyrosine kinase, a possible EGF receptor, eNOS is endothelial NO synthase, GC is guanylate cyclase, PKG is protein kinase G, and ROS is reactive oxygen species. The pathway supporting the transmission of signals from OR to mitoBK_{Ca} channels remains unstudied.

Conclusions

The data presented here provide evidence that transient ischemia/reperfusion increases in opioid peptides in myocardial tissues. Blockade of δ opioid receptors eliminates the cardioprotective effect of ischemia preconditioning both in vivo and in vitro. Inhibition of κ_1 opioid receptors eliminates the cardioprotective action of preconditioning in in vitro experiments. Agonists of μ, δ_1, δ_2, and κ_1 opioid receptors imitate the cardioprotective effect of preconditioning. Thus, there are grounds for the view that endogenous opioid peptides are triggers for ischemic preconditioning.

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