

Cardioprotective Properties of Opioid Receptor Agonists in Rats With Stress-Induced Cardiac Injury

E. S. PROKUDINA¹, L. N. MASLOV¹, N. V. NARYZHNAIA¹, S. YU. TSIBULNIKOV¹,
Y. B. LISHMANOV^{1,2}, J. E. MADIAS³, P. R. OELTGEN⁴

¹Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia, ²Laboratory of Nuclear Medicine, National Research Tomsk Polytechnic University, Tomsk, Russia, ³Icahn School of Medicine at Mount Sinai and the Division of Cardiology, Elmhurst Hospital Center, New York, New York, USA, ⁴Department of Pathology, University of Kentucky College of Medicine, Lexington, KY, USA

Received May 22, 2018

Accepted December 4, 2018

Epub Ahead of Print March 22, 2019

Summary

The objectives of this study were to investigate the role of endogenous opioids in the mediation of stress-induced cardiomyopathy (SIC), and to evaluate which opioid receptors regulate heart resistance to immobilization stress. Wistar rats were subjected to 24 h immobilization stress. Stress-induced heart injury was assessed by ^{99m}Tc-pyrophosphate accumulation in the heart. The opioid receptor (OR) antagonists (naltrexone, NxMB – naltrexone methyl bromide, MR 2266, ICI 174.864) and agonists (DALDA, DAMGO, DSLET, U-50,488) were administered intraperitoneally prior to immobilization and 12 h after the start of stress. In addition, the selective μ OR agonists PL017 and DAMGO were administered intracerebroventricularly prior to stress. Finally pretreatment with guanethidine was used. Naltrexone did not alter the cardiac ^{99m}Tc-PP accumulation in stressed rats. NxMB aggravated stress-induced cardiomyopathy ($P=0.005$) (SIC). The selective μ OR agonist DALDA, which does not cross the blood-brain barrier, completely prevented ($P=0.006$) SIC. The μ OR agonist DAMGO exhibited weaker effect than DALDA. The selective δ ligand (DSLET) and κ OR ligand (U-50,488) did not alter stress-induced ^{99m}Tc-pyrophosphate accumulation in the heart. Intracerebroventricular administration of the μ OR agonists aggravated SIC. Pretreatment with guanethidine abolished this effect ($P=0.01$). Guanethidine alone exhibited cardioprotective properties. A stimulation of central μ OR promotes an appearance of SIC. In contrast, stimulation of

peripheral μ OR contributes to an increase in cardiac tolerance to stress.

Key words

^{99m}Tc-pyrophosphate • Opioid receptors • Tako-tsubo syndrome • Stress • Cardiomyopathy

Corresponding author

L. N. Maslov, Laboratory of Experimental Cardiology, Federal State Budgetary Scientific Institution «Research Institute for Cardiology», Kyevskaya 111A, 634012 Tomsk, Russia. E-mail: maslov@cardio-tomsk.ru

Introduction

In the mid-seventies of the last century it was found that restraint stress could cause cardiomyopathy in pigs (Jönsson *et al.* 1975, Johansson *et al.* 1974), and food-shock stress could induce cardiomyopathy in rats (Miller and Mallov 1977). In 1990, stress-induced cardiomyopathy was described in humans by Sato *et al.* (1990). These authors called the condition tako-tsubo syndrome (TS) because the left ventricle of patients afflicted with this pathology during systole resembles the Japanese octopus fishing implement called “tako-tsubo” (Sato *et al.* 1990). Later, the existence of the disease was confirmed by other cardiologists (Pavin *et al.* 1997,

Tsuchihashi *et al.* 2001, Kurisu *et al.* 2003, Akashi *et al.* 2003). The incidence of TS was found to be increased as a result of improved diagnostic methods. According to Khera *et al.* (2016) from 2007 to 2012, the incidence of TS increased over 3-fold in the United States, as reflected in the relevant literature. Thus, in 2007, there were 52 cases per 1 million, and in 2012 already 178 cases per million hospitalized patients were reported (Khera *et al.* 2016). The rate of 28-day mortality in patients with TS is similar to mortality in patients with ST-segment elevation myocardial infarction (STEMI) (5.5 % vs. 5.7 %) (Stiermaier *et al.* 2016), while the rate of 1-year mortality in patients with TS and in patients with STEMI is 12.5 % vs. 9 %, respectively. The long-term mortality (during 3.8 ± 2.5 years) in patients with TS was significantly higher compared to the one of STEMI patients (24.7 % vs. 15.1 %) (Stiermaier *et al.* 2016). In-hospital mortality in the TS patients with cardiopulmonary failure is 18 % (El-Batrawy *et al.* 2017). This high mortality may be partially due to limitations of our knowledge about the pathogenesis of the disease, and as a result to the lack of a specific effective therapy of TS. In our opinion, animal studies could contribute important insights in the pathogenesis and management of TS.

Immobilization stress in rats has been found to promote the development of a state similar to TS (Ueyama *et al.* 2002, Ueyama *et al.* 2004). Although researchers have been studying TS already for the past 26 years, the pathogenesis of this disease remains a mystery in many ways. Currently, the focus is on activation of the adrenergic system in the pathogenesis of stress-induced cardiomyopathy (Chen *et al.* 2017, Dilsizian *et al.* 2017, Pelliccia *et al.* 2017, Sestini *et al.* 2017, Casey *et al.* 2017, Kido *et al.* 2017, Ceccacci *et al.* 2016, Chen *et al.* 2016, Christensen *et al.* 2016). Exogenous catecholamines can cause the TS (Casey *et al.* 2017, Kido *et al.* 2017, Elikowski *et al.* 2017, Nazir *et al.* 2017, Belliveau *et al.* 2016). Stress-induced cardiomyopathy (SIC) is often noted in patients with pheochromocytoma (Elikowski *et al.* 2017, Agrawal *et al.* 2017, Zhang *et al.* 2017, Y-Hassan *et al.* 2016). It is believed that SIC is an excessive activation of β -adrenergic receptors (Chen *et al.* 2017, Oras *et al.* 2017a, Oras *et al.* 2017b, Brunetti *et al.* 2016), so some authors mimic SIC by administration of toxic doses of isoproterenol (Oras *et al.* 2017a, Oras *et al.* 2017b, Sachdeva *et al.* 2014). This approach seems to us not quite correct, because in the case of using isoproterenol, other humoral factors that may be involved in the

mechanism of SIC origin are not considered. Many people find themselves in severe stressful situations but TS does not occur. We hypothesize that the endogenous peripheral opioid system plays a significant role in providing a deterrent mechanism to block TS from developing. We have previously shown that the 24-hour immobilization stress can cause accumulation of 99m Tc-pyrophosphate in rat myocardium (Lishmanov *et al.* 1997) that according to Miller and Mallov (1977) is an indicator of stress-induced myocardial injury. According to our data the ligands of opioid receptors can modulate stress heart damage (Lishmanov *et al.* 1997). The objectives of this study were to investigate the role of endogenous opioids in the mediation of stress-induced cardiomyopathy, and to evaluate which opioid receptors regulate heart resistance to immobilization stress.

Materials and Methods

Animals and research protocol

Male Wistar rats weighing 230-250 g were housed in groups of four rats per cage and allowed free access to tap water and a standard laboratory rat chow. Animals were kept in an air-conditioned room, where the temperature was maintained at 23 ± 1 °C, and the relative humidity was kept at 60-70 %. Animals were exposed to a 12 h day-night cycle.

Stress was induced by a 24-hour immobilization of animals in the supine position. The rats were fixed with adhesive tape for each limb thus reducing trauma related to the procedure. Naïve rats were used as control animals. They were not supplied with water and chow during immobilization stress. Animals were immobilized and injected intraperitoneally two times with a solution of 0.9 % NaCl (1 ml/kg) and were used as stress control. The first injection was administered at 9.00 a.m. and the second injection at 21.00 p.m. The study was approved by the Ethical Committee of the Cardiology Research Institute (approval number: 79, from 14.10.2016), and it conformed to the European Union Directive 2010/63/EU.

In most experiments, ligands of opioid receptors (OR) were administered intraperitoneally twice: 30 min before immobilization and 12 h after the start of exposure to the stress (Lishmanov *et al.* 1997). In addition μ OR agonists were administered intracerebroventricularly *via* a cannula implanted in advance. The preferential μ and κ OR antagonist naltrexone was administered at a dose of 0.5 mg/kg ($n=12$) (Thomas *et al.* 1998). Naltrexone methyl bromide (NxMB), an OR antagonist that does not

cross the blood-brain barrier (BBB), was used at a dose of 5 mg/kg (n=12) (Brown *et al.* 1983). The half-life of methylnaltrexone in the blood plasma is 7.6 h (Misra *et al.* 1987) and the half-life of naltrexone in various mammalian species is from 4 to 10 h (Crabtree 1984). Therefore, these drugs are capable of providing long-term blockade of opioid receptors. A preferential κ OR antagonist MR2266 ((-)2-(3-furyl methyl)-5,9-diethyl-2-hydroxy-6,7-benzomorphan) (Lahti *et al.* 1985) was administered at a dose of 5 mg/kg (n=12). The selective δ OR antagonist ICI 174.864 (N, N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH, where the Aib is α -aminoisobutyric acid) was administered at a dose of 2.5 mg/kg (n=12) (Dauge *et al.* 1988, Rebrova *et al.* 2001). The selective μ OR agonist that does not cross the BBB DALDA (H-Tyr-D-Arg-Phe-Lys-NH₂) (Roques *et al.* 1990, Samii *et al.* 1994) was given at a dose of 0.1 mg/kg (n=12) (Rebrova *et al.* 2001, Maslov *et al.* 2002). The selective μ OR agonist DAMGO (H-Tyr-D-Ala-Gly-N α -Me-Phe-Gly-ol) 20 was injected at a dose of 0.1 mg/kg (n=12) (Rebrova *et al.* 2001). The selective δ OR agonist DSLET (H-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH) 20 was administered at a dose of 0.1 mg/kg (n=12) (Rebrova *et al.* 2001). The selective κ_1 OR agonist (\pm)-U-50,488 (*trans*-(\pm)-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl] benzeneacetamide hydrochloride) (Lahti *et al.* 1985, Von Voigtlander *et al.* 1982) was used at a dose of 8 mg/kg (n=12) (Von Voigtlander *et al.* 1982).

In addition, DAMGO was administered intracerebroventricularly and the selective μ OR agonist PL017 (Tyr-Pro-N α -Me-Phe-D-Pro-NH₂) (Chang *et al.* 1983) was infused intracerebroventricularly both at a dose of 20 μ g two times: 30 min before immobilization and 12 h after immobilization (Chang *et al.* 1983). Guanethidine monosulfate, a compound which depletes peripheral storage of endogenous catecholamines (Maitre *et al.* 1971), was used at a dose of 50 mg/kg subcutaneously every day during 3 days (Maslov *et al.* 2009). The last injection of guanethidine was performed 24 h before immobilization. Each group included 12 animals. A total of 432 rats were included in the study. Control (naive) group included 96 animals.

Measurement of ^{99m}Tc-pyrophosphate accumulation

The evaluation of the extent of the stress-induced cardiac damage was studied by the assessment of the level of myocardial accumulation of radioactive ^{99m}Tc-pyrophosphate (^{99m}Tc-PP), which was administered intravenously in a dose of 150 MBq/kg 30 min after

cessation of immobilization (Miller and Mallov 1977). The animals were decapitated under ethyl ether anesthesia 100 min after the injection. Hearts were removed from thorax and perfused through aorta with cold physiological saline (10 ml, 10 °C). Registration of radioactivity was measured by the γ counter RIS-A1-E "Doscalibrator" Amplitude Company, (Third Zapadnyi 15, Zelenograd, Moscow, Russia. The accumulation of ^{99m}Tc-PP in the myocardial tissue was expressed as a percent of administered dose per 1 g of heart tissue as % of total dose/g weight of heart x 100.

Surgical procedure

The cannula implantation was performed as described previously (Lishmanov *et al.* 2009). Five to 7 days before the induction of stress, a cannula, consisting of a 30 gauge stainless steel needle (SFM Hospital Products, Berlin, Germany), was inserted into the lateral cerebral ventricle of rats and was fixed in the skull by dental cement. This procedure was undertaken in surgery and in experimental procedures under pentobarbital anesthesia (50 mg/kg), and was facilitated by the use of a stereotaxic apparatus (SEZh-5; Constructor Company, Acadimika Bogomoltsa 4, Kiev, Ukraine). The cannula was inserted at the following coordinates from the bregma: AP -1.5 mm, +2.0 mm; V -3.5 mm. The place of injection in the lateral cerebral ventricle was confirmed by injecting methylene blue dye through the cannula at the end of the experiment. At the end of the experiment, the brain was removed, placed in formalin and later sectioned. Correct placement of the cannula was confirmed by the presence of dye in the cerebroventricular system in all rats.

Pharmacological agents

Naltrexone, U-50,488 were purchased from Sigma-Aldrich (USA). Naltrexone methyl bromide and MR2266 were synthesized by Boehringer Ingelheim KG (Ingelheim am Rhein, Germany). Peptides ICI 174.864, DAMGO, DSLET, PL017 were synthesized by Chiron Mimotopes Peptide Systems (San Diego, USA). Peptide DALDA was synthesized by Laboratory of Chemical Biology and Peptide Research, Clinical Research Institute of Montreal, Montreal, Quebec, Canada. Guanethidine was synthesized by the International Laboratory (San Bruno, CA, USA) and purchased from Advanced Technology and Industrial Co. (Hong Kong, China).

Statistical analysis

Results are expressed as mean \pm SEM from indicated number of experiments. Statistical comparison of means between groups was made by one-way ANOVA. The assumption of normality distribution data has been tested by Shapiro-Wilk (SW) normality test. Values exceeding the 95 % probability limits ($P<0.05$) were considered significant.

Results

As shown in Figure 1, immobilization stress caused an increase in the 99m Tc-PP accumulation in the myocardium by 1.5 fold ($P=0.001$) (~50 %). Pretreatment with naltrexone did not alter the cardiac 99m Tc-PP accumulation in stressed rats (Fig. 1). On the contrary, injection of NxMB contributed to an increase in stress-evoked 99m Tc-PP uptake by the heart ($P=0.005$) (Fig. 1). It should be noted that the administration of naltrexone or NxMB had no effect on the accumulation of 99m Tc-PP in the myocardium of naïve rats (Fig. 1). Pretreatment with DALDA caused a decrease in the stress-induced accumulation of 99m Tc-PP in the myocardium by 1.8 fold ($P=0.006$) (Fig. 2a). The selective μ OR agonist DAMGO resulted in a weaker similar effect, reducing the stress induced 99m Tc-PP heart uptake only by 1.36 fold ($P=0.01$) (Fig. 2a). The selective μ OR agonists had no effect on cardiac 99m Tc-PP accumulation in naïve rats. In contrast, intracerebroventricular administration of DAMGO and PL017 enhanced the 99m Tc-PP accumulation in the heart after immobilization ($P=0.01$) (Fig. 2b). It should be noted that the intracerebroventricular administration of selective μ OR agonists had no effect on the cardiac 99m Tc-PP accumulation in naïve rats. As shown in Figure 3, the selective δ OR antagonist ICI 174.864 and the preferential κ OR antagonist MR2266 did not alter the cardiac 99m Tc-PP accumulation in naïve and stressed rats. Also the selective δ OR agonist DSLET and the selective κ OR agonist U-50,488 had no effect on the 99m Tc-PP uptake by the heart in both groups of animals (Fig. 4). Pretreatment with guanethidine contributed to a decrease in the cardiac 99m Tc-PP accumulation in stressed rats, compared with stress control group, and attenuated intracerebroventricular DAMGO-induced 99m Tc-PP accumulation in stressed rats ($P=0.01$) (Fig. 5). Finally, guanethidine did not change the cardiac 99m Tc-PP accumulation in naïve rats.

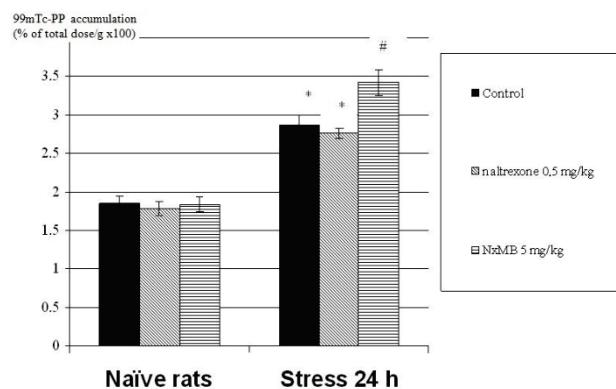


Fig. 1. Effect of the administration of 2 non-selective opioid receptor antagonists on the immobilization stress induced myocardial injury measured by the level of accumulation of 99m Tc pyrophosphate. * Significant difference in comparison with naïve animals. # Significant differences compared to control stress.

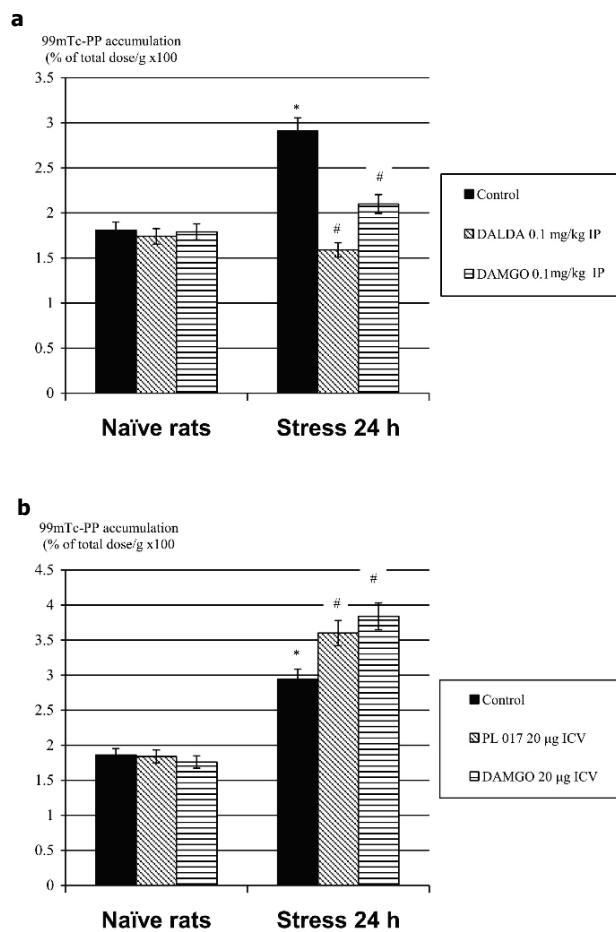


Fig. 2. (a) Effect of the intraperitoneal administration of 2 peptide μ opioid receptor agonists on the immobilization stress induced myocardial injury measured by the level of accumulation of 99m Tc pyrophosphate. * Significant difference in comparison with naïve animals. # Significant differences compared to control stress. (b) Effect of the intracerebroventricular administration of 2 peptide μ opioid receptor agonists on the immobilization stress induced myocardial injury measured by the level of accumulation of 99m Tc pyrophosphate. * Significant difference in comparison with naïve animals. # Significant differences compared to control stress.

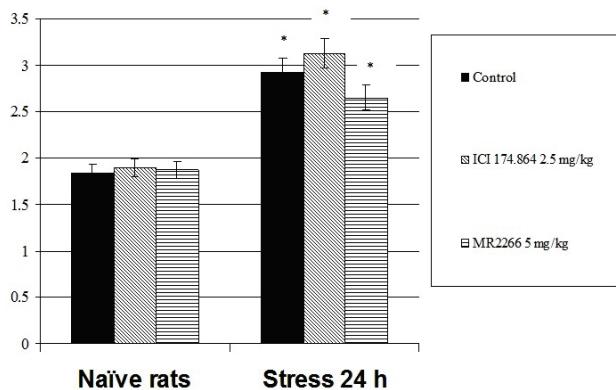


Fig. 3. Effect of administration of δ and κ opioid receptor antagonists on the immobilization stress induced myocardial injury measured by the level of accumulation of ^{99m}Tc -PP pyrophosphate. * Significant difference in comparison with naïve animals.

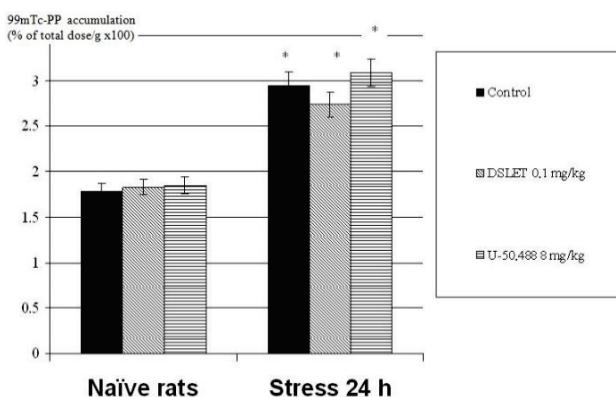


Fig. 4. Effect of administration of selective δ and κ opioid receptor agonists on the immobilization stress induced myocardial injury measured by the level of accumulation of ^{99m}Tc pyrophosphate. * Significant difference in comparison with naïve animals.

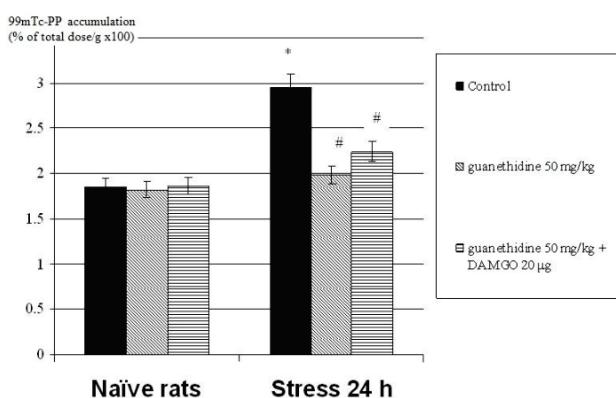


Fig. 5. Effect of subcutaneous administration of guanethidine and intracerebroventricular administration of DAMGO on the immobilization stress induced myocardial injury measured by the level of accumulation of ^{99m}Tc pyrophosphate. * Significant difference in comparison with naïve animals. # Significant differences compared to control stress.

Discussion

Our results indicate that the immobilization stress causes damage to the heart. Our data are consistent with the results of other investigators (Miller and Mallov 1977) who also observed an enhancement of ^{99m}Tc -PP accumulation in the myocardium of food-shock stressed rats. Pretreatment with guanethidine prevented the stress-induced ^{99m}Tc -PP uptake by the heart of the stressed rats that indicated the involvement of endogenous catecholamines in the pathogenesis of SIC. In addition, these data indicate that our rat model of SIC is similar to TS because endogenous catecholamines are involved in the appearance of both immobilization induced cardiomyopathy and also TS (Riester *et al.* 2015, Smeijers *et al.* 2015, Sharkey *et al.* 2015, Nunez-Gil *et al.* 2015).

We found that administration of NxMB, which does not penetrate into the brain from the bloodstream, aggravated SIC. On the other hand, naltrexone, which crosses the BBB, prevented the emergence of SIC. These data indicated that endogenous agonists of central ORs are involved in the pathogenesis of SIC. The endogenous agonists of peripheral ORs prevented the occurrence of SIC. Endogenous agonists κ and δ OR perhaps are not involved in the development of this cardiomyopathy, since antagonists and agonists of these ORs had no effect on the stress induced ^{99m}Tc -PP accumulation in the myocardium. It is well known that naltrexone and NxMB exhibit a high affinity for μ OR. Therefore, it can be hypothesized that central and peripheral μ ORs are involved in the regulation of cardiac tolerance to stress but their role in regulating the cardiac tolerance to stress is different. Activation of the central μ OR aggravates the pathologic effect of stress and a stimulation of the peripheral μ OR has a cardioprotective effect. Therefore, NxMB exacerbates the pathogenic effect of stress. Naltrexone blocks both central and peripheral μ ORs thereby excluding their involvement in the regulation of cardiac tolerance to impact of stress.

We decided to test this hypothesis using the selective μ OR agonist DALDA, which cannot cross the BBB (Samii *et al.* 1994), and DAMGO, which exhibits antinociceptive effect (hot-plate test in mice) at a dose of 2.5 mg/kg probably due to the stimulation of central μ OR. The antinociceptive effect in hot-plate test indirectly indicates stimulation of the central opioid receptors (Delay-Goyet *et al.* 1991). This opioid did not exhibit antinociceptive effect at a dose of 1.25 mg/kg in

this test (Delay-Goyet *et al.* 1991). We used DAMGO at a dose of 0.1 mg/kg to avoid activation of central OR. Both opioid peptides exhibit similar affinity to μ OR (Schiller *et al.* 1989). The single most important difference between these opioids is the permeability to the BBB. We found that DALDA showed a strong cardioprotective effect in the stressed rats, while the cardioprotective effect of DAMGO was weaker, probably because small quantities of DAMGO can cross the BBB, and partially occupy central μ OR. These results are in agreement with our previously published data (Lishmanov *et al.* 2017).

In further experiments, we explored whether the activation of the central μ OR would affect the heart resistance to stress. It turned out that intracerebroventricular administration of DAMGO, or the selective μ OR agonist PL017 aggravated the stress-induced injury to the heart. Pretreatment with guanethidine completely eliminated the negative effect of intracerebroventricular DAMGO administration. This result suggested an involvement of the sympathetic nervous system (SNS) in the mediation of stress-induced myocardial injury, and our hypothesis is in agreement with data of other investigators (Hassen and Feuerstein 1987, Kiritsy-Roy *et al.* 1989, Yamauchi *et al.* 1997) and our previously published data (Lishmanov *et al.* 2017).

In 1987, Hassen and Feuerstein found that the stimulation of μ OR in *n. tractus solitarius* leads to activation of the SNS. In 1989, Kiritsy-Roy *et al.* experiments with awake rats showed that intracerebroventricular administration of selective μ OR agonist DAMGO or δ OR agonist DPDPE leads to a 2 to 3 fold increase in the plasma level of norepinephrine, and increase in plasma epinephrine concentration by several ten folds. Maximum stimulation of SNS was achieved with the administration of 5 nM of DAMGO and 125 nM of DPDPE, with the latter compound having no effect on catecholamine levels when administered at a dose of 5 nM (Kiritsy-Roy *et al.* 1989). In 1997, Yamauchi *et al.* found that the intracerebroventricular administration of β -endorphin (preferential μ and δ OR agonist R) causes an increase in blood plasma epinephrine and norepinephrine levels in rats. Naloxone (2 mg/kg intravenously) completely eliminated this effect of β -endorphin. These data demonstrate that occupancy of central μ OR by agonists can promote an activation of SNS. In contrast there are data that demonstrate following occupancy of peripheral μ OR by agonists results in a limitation of norepinephrine release from sympathetic

nerve terminals in the heart (Ledda *et al.* 1982, Ensinger *et al.* 1984, Von Kugelgen *et al.* 1985, Fuder *et al.* 1986, Szabo *et al.* 1986), and epinephrine release from the adrenal glands is observed (Chen *et al.* 1989). However, most publications indicate that the limitation of norepinephrine release from peripheral sympathetic terminals is a result of presynaptic δ and κ OR activation (Von Kugelgen *et al.* 1985, Fuder *et al.* 1986, Szabo *et al.* 1986), and thus it is unclear why these receptor agonists (DSLET and U-50,488) had no effect on SIC. Furthermore, it was found that κ OR agonists can inhibit synthesis of catecholamines in chromaffin cells (Takekoshi *et al.* 2000). Opioid receptors have been found in chromaffin cells (Saiani *et al.* 1982, Abood *et al.* 1995, Kampa *et al.* 1999) primarily consisting of δ (Abood *et al.* 1995) or κ OR (Kampa *et al.* 1999). Kampa *et al.* (1999) demonstrated that human pheochromocytoma cells contain κ_1 OR, fewer κ_2 OR and minimal binding capacity for δ and μ OR agonists sites. It remains unclear why exactly the activation of peripheral μ opioid receptors increased cardiac tolerance to stress. In conclusion, it should be noted that in the regulation of heart tolerance to the impact of ischemia and reperfusion an important role is played by μ , δ , κ ORs (Abood *et al.* 1995), and in regulation of cardiac tolerance to the stress only μ OR are involved according to our data. The reason for such differences in the functional role of ORs remains unclear.

Conclusions

Naltrexone enhanced the cardiac tolerance to the immobilization stress. Naltrexone methyl bromide aggravated stress-induced cardiomyopathy. The selective μ OR agonist DALDA, which does not cross the BBB, completely prevented stress-induced heart injury. The selective μ OR agonist DAMGO, which is capable of crossing the BBB, exhibited weaker effect than DALDA. The selective δ and κ OR ligands did not alter the stress induced 99m Tc-pyrophosphate accumulation in the heart. Intracerebroventricular administration of the selective μ OR agonists aggravated the stress-induced heart injury. Pretreatment with guanethidine abolished the noxious effects of the immobilization stress. Guanethidine administered alone exhibited cardioprotective properties. Thus, stimulation of central μ OR mediates the emergence of stress cardiomyopathy. In contrast, stimulation of peripheral μ OR contributes to an increase in cardiac tolerance to stress. Overall, these results

support our hypothesis that opioid-mediated cardioprotection is mediated via peripheral μ OR activation.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

The authors are grateful to Dr. Kevin Gormley (Division of Neuroscience & Behavioral Research, NIDA NIH, Bethesda, USA) for providing the peptides (DAMGO,

DSLET, ICI 174.864, PL017). The authors are grateful to Professor P. W. Schiller (Clinical Research Institute of Montreal, Montreal, Quebec, Canada) for kindly granting DALDA. The authors are grateful to Drs. P Veerhoff and Dr. Duttman (Boehringer Ingelheim KG, Ingelheim am Rhein, Germany) who kindly donated the naltrexone methyl bromide and MR2266. This study was funded by Russian Science Foundation (grant 18-75-00001). The section dedicated to naltrexone is framed within the framework of state assignments AAAA-A15-115120910024-0.

References

- ABOOD ME, TAO Q: Characterization of a delta opioid receptor in rat pheochromocytoma cells. *J Pharmacol Exp Ther* **274**: 1566-1573, 1995.
- AGRAWAL S, SHIRANI J, GARG L, SINGH A, LONGO S, LONGO A, FEGLEY M, STONE L, RAZAVI M, RADOIANU N, NANDA S: Pheochromocytoma and stress cardiomyopathy: Insight into pathogenesis. *World J Cardiol* **9**: 255-260, 2017.
- AKASHI YJ, NAKAZAWA K, SAKAKIBARA M, MIYAKE F, KOIKE H, SASAKA K: The clinical features of takotsubo cardiomyopathy. *QJM* **96**: 563-573, 2003.
- BELLIVEAU D, DE S: Reverse takotsubo cardiomyopathy following exogenous epinephrine administration in the early postpartum period. *Echocardiography* **33**: 1089-1091, 2016.
- BROWEN DR, ROBERTSON MJ, GOLDBERG LI: Reversal of morphine-induced catalepsy in the rat by narcotic antagonists and their quaternary derivatives. *Neuropharmacology* **22**: 317-321, 1983.
- BRUNETTI ND, SANTORO F, DE GENNARO L, CORREALE M, GAGLIONE A, DI BIASE M: Drug treatment rates with beta-blockers and ACE-inhibitors/angiotensin receptor blockers and recurrences in takotsubo cardiomyopathy: A meta-regression analysis. *Int J Cardiol* **214**: 340-342, 2016.
- CASEY RT, CHALLIS BG, PITFIELD D, MAHROOF RM, JAMIESON N, BHAGRA CJ, VUYLSTEKE A, PETTIT SJ, CHATTERJEE KC: Management of an acute catecholamine-induced cardiomyopathy and circulatory collapse: a multidisciplinary approach. *Endocrinol Diabetes Metab Case Rep* **2017**: pii: 17-0122, 2017.
- CECCACCI A, MANCONE M, CALCAGNO S, DE VINCENTIS G, SARDELLA G, FEDELE F: Role of MIBG scintigraphy in reverse Tako-tsubo cardiomyopathy: Confirming a pathophysiologic hypothesis. *Int J Cardiol* **223**: 54-55, 2016.
- CHANG KJ, WEI E, KILLIAN A, CHANG JK: Potent morphiceptin analogs: structure activity relationships and morphine-like activities. *J Pharmacol Exp Ther* **227**: 403-408, 1983.
- CHEN W, DILSIZIAN V: Cardiac sympathetic disturbance in takotsubo cardiomyopathy: primary etiology or a compensatory response to heart failure? *JACC Cardiovasc Imaging* **9**: 991-993, 2016.
- CHEN W, DILSIZIAN V: Exploring the pathophysiology of takotsubo cardiomyopathy. *Curr Cardiol Rep* **19**: 53, 2017.
- CHEN YM, DIXON WR, WAKADE AR: The effect of etorphine on the secretion of endogenous catecholamines and total tritium evoked by nerve- and acetylcholine-stimulation in perfused rat adrenal glands. *Life Sci* **44**: 167-174, 1989.
- CHRISTENSEN TE, BANG LE, HOLMVANG L, SKOVGAARD DC, OTURAI DB, SØHOLM H, THOMSEN JH, ANDERSSON HB, GHOTBI AA, IHLEMANN N, KJAER A, HASBAK P: ^{123}I -MIBG scintigraphy in the subacute state of takotsubo cardiomyopathy. *JACC Cardiovasc Imaging* **9**: 982-990, 2016.
- CRABTREE BL: Review of naltrexone, a long-acting opiate antagonist. *Clin Pharm* **3**: 273-280, 1984.

- DAUGE V, ROSSIGNOL P, ROQUES BP: Comparison of the behavioural effects induced by administration in rat nucleus accumbens or nucleus caudatus of selective μ and δ opioid peptides or kelatorphan an inhibitor of enkephalin-degrading-enzymes. *Psychopharmacology (Berl)* **96**: 343-352, 1988.
- DELAY-GOYET P, RUIZ-GAYO M, BAAMONDE A, GACEL G, MORGAT JL, ROQUES BP: Brain passage of BUBU, a highly selective and potent agonist for delta opioid receptors: in vivo binding and mu versus delta receptors occupancy. *Pharmacol Biochem Behav* **38**: 155-162, 1991.
- EL-BATTRAWY I, LANG S, ANSARI U, SATTLER K, BEHNES M, SCHRAMM K, FASTNER C, TÜLÜMEN E, ZHOU X, HOFFMANN U, BORGGREFE M, AKIN I: Incidence and prognostic relevance of cardiopulmonary failure in takotsubo cardiomyopathy. *Sci Rep* **7**: 14673, 2017.
- ELIKOWSKI W, MAŁEK-ELIKOWSKA M, KAROŃ J, MROŻIŃSKA M, BASZKO A, HORBACKA K: Takotsubo cardiomyopathy after intravenous epinephrine administration following cardiac arrest provoked by pneumoperitoneum - a case report. *Pol Merkur Lekarski* **42**: 165-169, 2017.
- ENSINGER H, HEDLER L, SCHURR C, STARKE K: Ethylketocyclazocine decreases noradrenaline release and blood pressure in the rabbit at a peripheral opioid receptor. *Naunyn Schmiedebergs Arch Pharmacol* **328**: 20-23, 1984.
- FUDER H, BUDER M, RIERS HD, ROTHACHER G: On the opioid receptor subtype inhibiting the evoked release of ^3H -noradrenaline from guinea-pig atria in vitro. *Naunyn Schmiedebergs Arch Pharmacol* **332**: 148-155, 1986.
- HASSEN AN, FEUERSTEIN G: μ -Opioid receptors in NTS elicit pressor responses via sympathetic pathways. *Am J Physiol* **252**: H156-H162, 1987.
- JOHANSSON G, JONSSON L, LANNEK N, BLOMGREN L, LINDBERG P, POUPA O: Severe stress-cardiopathy in pigs. *Am Heart J* **87**: 451-457, 1974.
- JÖNSSON L, JOHANSSON G, LANNEK N, LINDBERG P, POUPA O: Histochemical and electron microscopic studies of acute cardiomyopathy induced by restraint stress in pigs. *Recent Adv Stud Cardiac Struct Metab* **6**: 461-470, 1975.
- KAMPA M, MARGIORIS AN, HATZOGLOU A, DERMITZAKI I, DENIZOT A, HENRY JF: κ_1 -Opioid binding sites are the dominant opioid binding sites in surgical specimens of human pheochromocytomas and in a human pheochromocytoma (KAT45) cell line. *Eur J Pharmacol* **364**: 255-262, 1999.
- KHERA R, LIGHT-MCGROARY K, ZAHR F, HORWITZ PA, GIROTRA S: Trends in hospitalization for takotsubo cardiomyopathy in the United States. *Am Heart J* **172**: 53-63, 2016.
- KIDO K, GUGLIN M: Drug-induced takotsubo cardiomyopathy. *J Cardiovasc Pharmacol Ther* **22**: 552-563, 2017.
- KIRITSY-ROY JA, MARSON L, VAN LOON GR: Sympathoadrenal, cardiovascular and blood gas responses to highly selective mu and delta opioid peptides. *J Pharmacol Exp Ther* **251**: 1096-1103, 1989.
- KURISU S, SATO H, KAWAGOE T, ISHIHARA M, SHIMATANI Y, NISHIOKA K: Tako-tsubo-like left ventricular dysfunction with ST-segment elevation: a novel cardiac syndrome mimicking acute myocardial infarction. *Am Heart J* **143**: 448-455, 2002.
- LAHTI RA, MICHELSON MM, MCCALL JM, VON VOIGTLANDER PF: $[^3\text{H}]$ U-69593 a highly selective ligand for the opioid κ receptor. *Eur J Pharmacol* **109**: 281-284, 1985.
- LEDDA F, MANTELLI L: Possible presynaptic inhibitory effect of etorphine on synaptic nerve terminals of guinea-pig heart. *Eur J Endocrinol* **85**: 247-250, 1982.
- LISHMANOV YB, MASLOV LN, NARYZHNAVA NV: Cardioprotective effects of stimulation of peripheral μ -opiate receptors and the role of opiateergic mechanisms in the pathogenesis of stress-induced heart damage. *Bull Exp Biol Med* **123**: 239-241, 1997.
- LISHMANOV YB, MASLOV LN, UGDYZHEKOVA DS: Participation of central and peripheral κ_1 and κ_2 opioid receptors in arrhythmogenesis. *Clin Exp Pharmacol Physiol* **26**: 716-723, 1999.
- LISHMANOV YB, TSIBUL'NIKOV SY, NARYZHNAVA NV, KOROBON MV, MASLOV LN: The role of endogenous opioid system in the regulation of heart tolerance to stress-induced damage. *Bull Exp Biol Med* **163**: 25-27, 2017.
- MAITRE L, STAHELIN M: Guanethidine uptake and noradrenaline depletion in noradrenaline storage particles of the rat heart. *Biochem Pharmacol* **20**: 1233-1242, 1971.

- MASLOV LN, LISHMANOV YB, OELTGEN PR, BARZAKH EI, KRYLATOV AV, GOVINDASWAMI M: Activation of peripheral δ_2 opioid receptors increases cardiac tolerance to ischemia/reperfusion injury: Involvement of protein kinase C, NO-synthase, K_{ATP} channels and the autonomic nervous system. *Life Sci* **84**: 657-663, 2009.
- MASLOV LN, KRYLATOV AV, NARYZHAI A NV, SOLENKOVA NV, LISHMANOV AIU, BOGOMAZ SA, GROSS GJ, STEFANO JB, LOKTIUSHINA BA: Interactions of peripheral mu-opioid receptors and K_{ATP} -channels in regulation of cardiac electrical stability in ischemia, reperfusion, and postinfarction cardiosclerosis. *Ross Fiziol Zh Im I M Sechenova* **88**: 842-850, 2002.
- MILLER DG, MALLOV S: Quantitative determination of stress-induced myocardial damage in rats. *Pharmacol Biochem Behav* **7**: 139-145, 1977.
- MISRA AL, PONTANI RB, VADLAMANI NL: Intravenous kinetics and metabolism of [15,16- 3 H]naltrexonium methiodide in the rat. *J Pharm Pharmacol* **39**: 225-227, 1987.
- NAZIR S, LOHANI S, TACHAMO N, GHIMIRE S, POUDEL DR, DONATO A: Takotsubo cardiomyopathy associated with epinephrine use: A systematic review and meta-analysis. *Int J Cardiol* **229**: 67-70, 2017.
- NUNEZ-GIL IJ, BERNARDO E, FELTES G, ESCANED J, MEJÍA-RENTERÍA HD, DE AGUSTÍN JA: Platelet function in Takotsubo cardiomyopathy. *J Thromb Thrombolysis* **39**: 452-458, 2015.
- ORAS J, REDFORS B, ALI A, ALKHOURY J, SEEMAN-LODDING H, OMEROVIC E, RICKSTEN SE: Early treatment with isoflurane attenuates left ventricular dysfunction and improves survival in experimental Takotsubo. *Acta Anaesthesiol Scand* **61**: 399-407, 2017a.
- ORAS J, REDFORS B, ALI A, LUNDGREN J, SIHLBOM C, THORSELL A, SEEMAN-LODDING H, OMEROVIC E, RICKSTEN SE: Anaesthetic-induced cardioprotection in an experimental model of the Takotsubo syndrome - isoflurane vs. propofol. *Acta Anaesthesiol Scand* **61**: 309-321, 2017b.
- PAVIN D, LE BRETON H, DAUBERT C: Human stress cardiomyopathy mimicking acute myocardial syndrome. *Heart* **78**: 509-511, 1997.
- PELLICCIA F, KASKI JC, CREA F, CAMICI PG: Pathophysiology of takotsubo syndrome. *Circulation* **135**: 2426-2441, 2017.
- REBROVA TY, MASLOV LN, LISHMANOV AY, TAM SV: Stimulation of mu and delta-opiate receptors and tolerance of isolated heart to oxidative stress: the role of NO-synthase. *Biochemistry (Mosc)* **66**: 422-428, 2001.
- RIESTER A, WEISMANN D, QUINKLER M, LICHTENAUER UD, SOMMEREY S, HALBRITTER R: Life-threatening events in patients with pheochromocytoma. *Eur J Endocrinol* **173**: 757-764, 2015.
- ROQUES BP, GACEL G, DAUGE V, BAAMONDE A, CALENCO G, TURCAUD S: Novel approaches in the development of new analgesics. *Neurophysiol Clin* **20**: 369-387, 1990.
- SACHDEVA J, DAI W, KLONER RA: Functional and histological assessment of an experimental model of Takotsubo's cardiomyopathy. *J Am Heart Assoc* **3**: e000921, 2014.
- SAIANI L, GUIDOTTI A: Opiate receptor-mediated inhibition of catecholamine release in primary cultures of bovine adrenal chromaffin cells. *J Neurochem* **39**: 1669-1676, 1982.
- SAMII A, BICKEL U, STROTH U, PARTRIDGE WM: Blood-brain barrier transport of neuropeptides: analysis with a metabolically stable dermorphin analogue. *Am J Physiol* **267**: E124-E131, 1994.
- SATO H, TATEISHI H, UCHIDA T, ISHIHARA M, SHIMATANI Y, NISHIOKA K: Tako-tsubo-like left ventricular dysfunction due to multivessel coronary spasm. In: *Clinical Aspect of Myocardial Injury: From Ischemia to Heart Failure*. KODAMA K, HAZE K, HON M (eds), Tokyo: Kagakuyourosha, 1990, pp 56-64.
- SCHILLER PW, NGUYEN TM-D, LEMIEUX C: Two new families of opioid peptide analogs displaying extraordinary μ -receptor selectivity and preference for either peripheral or central sites. In: *Advances in the Biosciences*. London: Pergamon Press, 1989, pp 85-88.
- SESTINI S, PESTELLI F, LEONCINI M, BELLANDI F, MAZZEO C, MANSI L, CARRIO I, CASTAGNOLI A: The natural history of takotsubo syndrome: a two-year follow-up study with myocardial sympathetic and perfusion G-SPECT imaging. *Eur J Nucl Med Mol Imaging* **44**: 267-283, 2017.

- SHARKEY SW, McALLISTER N, DASSENKO D, LIN D, HAN K, MARON BJ: Evidence that high catecholamine levels produced by pheochromocytoma may be responsible for tako-tsubo cardiomyopathy. *Am J Cardiol* **115**: 1615-1618, 2015.
- SMEIJERS L, SZABÓ BM, VAN DAMMEN L, WONNINK W, JAKOBS BS, BOSCH JA: Emotional, neurohormonal, and hemodynamic responses to mental stress in Tako-Tsubo cardiomyopathy. *Am J Cardiol* **115**: 1580-1586, 2015.
- STIERMAIER T, MOELLER C, OEHLER K, DESCH S, GRAF T, EITEL C: Long-term excess mortality in takotsubo cardiomyopathy: predictors, causes and clinical consequences. *Eur J Heart Fail* **18**: 650-656, 2016.
- SZABÓ B, HEDLER L, ENSINGER H, STARKE K: Opioid peptides decrease noradrenaline release and blood pressure in the rabbit at peripheral receptors. *Naunyn Schmiedebergs Arch Pharmacol* **332**: 50-56, 1986.
- TAKEKOSHI K, ISHII K, KAWAKAMI Y, ISOBE K, NAKAI T: κ -Opioid inhibits catecholamine biosynthesis in PC12 rat pheochromocytoma cell. *FEBS Lett* **477**: 273-277, 2000.
- THOMAS JB, ZHENG X, MASCARELLA SW, ROTHMAN RB, DERSCH CM, PARTILLA JS: N-Substituted 9 β -methyl-5-(3-hydroxyphenyl)morphans are opioid receptor pure antagonists. *J Med Chem* **41**: 4143-4149, 1998.
- TSUCHIHASHI K, UESHIMA K, UCHIDA T, OH-MURA N, KIMURA K, OWA M: Transient left ventricular apical ballooning without coronary artery stenosis: a novel heart syndrome mimicking acute myocardial infarction: Angina Pectoris-Myocardial Infarction Investigations in Japan. *J Am Coll Cardiol* **38**: 11-18, 2001.
- UEYAMA T, KASAMATSU K, HANO T, YAMAMOTO K, TSURUO Y, NISHIO I: Emotional stress induces transient left ventricular hypocontraction in the rat via activation of cardiac adrenoceptors: a possible animal model of 'tako-tsubo' cardiomyopathy. *Circ J* **66**: 712-713, 2002.
- UEYAMA T: Emotional stress-induced Tako-tsubo cardiomyopathy: animal model and molecular mechanism. *Ann N Y Acad Sci* **1018**: 437-444, 2004.
- VON KUGELGEN I, ILLESS P, WOLF D, STARKE K: Presynaptic inhibitory opioid κ - and δ -receptors in a branch of the rabbit ileocolic artery. *Eur J Pharmacol* **118**: 97-103, 1985.
- VON VOIGTLANDER PF, LEWIS RA: U-50,488, a selective kappa opioid agonist: comparison to other reputed kappa agonists. *Prog Neuropsychopharmacol Biol Psychiatry* **6**: 467-470, 1982.
- YAMAUCHI N, SHIBASAKI T, WAKABAYASHI I, DEMURA H: Brain β -endorphin and other opioids are involved in restraint stress-induced stimulation of the hypothalamic-pituitary-adrenal axis, the sympathetic nervous system, and the adrenal medulla in the rat. *Brain Res* **777**: 140-146, 1997.
- Y-HASSAN S: Clinical features and outcome of pheochromocytoma-induced takotsubo syndrome: analysis of 80 published cases. *Am J Cardiol* **117**: 1836-1844, 2016.
- ZHANG R, GUPTA D, ALBERT SG: Pheochromocytoma as a reversible cause of cardiomyopathy: Analysis and review of the literature. *Int J Cardiol* **249**: 319-323, 2017.