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

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Summary

Purpose 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. *Methods* 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. *Results* 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. *Conclusions* 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, takotsubo syndrome, stress, cardiomyopathy

1. 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, Mallov, 1977). In 1990, stress-induced cardiomyopathy was described in humans by Sato et. al. (Sato H *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. (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-Battrawy *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; 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*. 2017ab; Brunetti *et al*. 2016), so some authors mimic SIC by administration of

toxic doses of isoproterenol (Oras *et al*. 2017ab; 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, Mallov (Miller, 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.

2. Materials and Methods

2.1. 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 which 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) (Browen *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-dially-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-NH_2) (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-Nα-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 (±)-U-50,488 (*trans*-(±)-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-Nα-Me-Phe-D-Pro-NH_2) (Chang *et al*. 1983) was infused intracerebroventricularly both at a dose of 20 μg 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.

2.2. 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, Mallov, 1977]. The animals were decapitated under ethyl ether anesthesia 100 minutes 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.

2.3. 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.

2.4. Pharmacological agents

Naltrexone, U-50,488 were purchased from Sigma-Aldrich (USA). Naltrexone methyl bromide and MR2266 were synthesized by Boehringer Inglheim KG (Inglheim 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).

2.5. Statistical analysis

Results are expressed as mean ± 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.

3. 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, DALDA 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.

4. Discussion

Our results indicate that the immobilization stress causes damage to the heart. Our data are consistent with the results of other investigators [Miller, 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, 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 (Hassen, Feuerstein 1987) found that the stimulation of μ OR in n. tractus solitarius leads to activation of the SNS. In 1989, Kiritsy-Roy et al. (Kiritsy-Roy *et al*. 1989) 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. (Yamauchi *et al*. 1997) 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 (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.

5. 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: The authors declare that they have no conflict of interest.

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Figure 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

Figure 2a. 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

Figure 2b. 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

Figure 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 pyrophosphate
* Significant difference in comparison with naïve animals

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

Figure 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

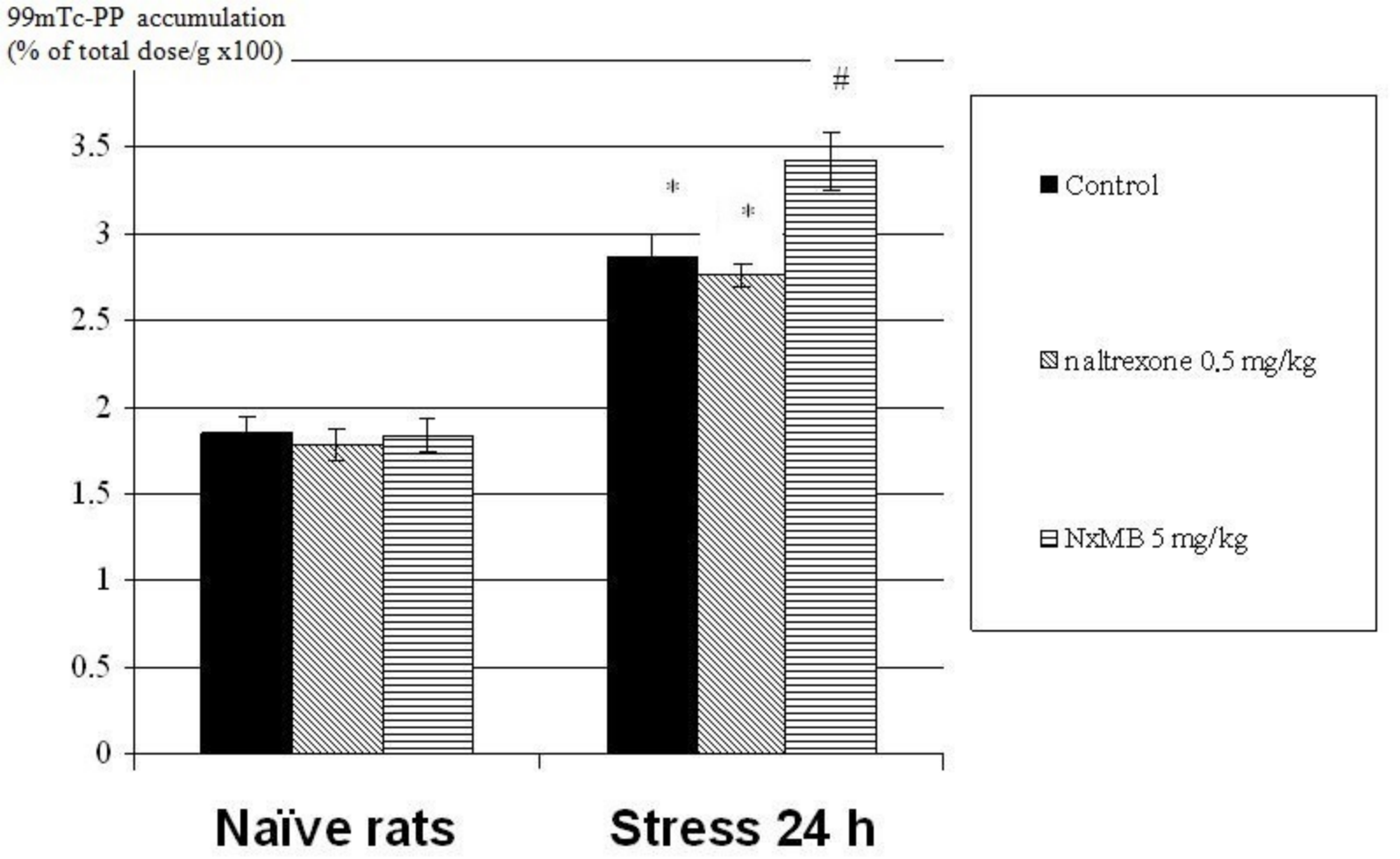
99mTc-PP accumulation
(% of total dose/g x100)
3.5
3
2.5
2
1.5
1
0.5
0
#
*
*
Control
naltrexone 0.5 mg/kg
NxMB 5 mg/kg
Naïve rats
Stress 24 h

Fig.1

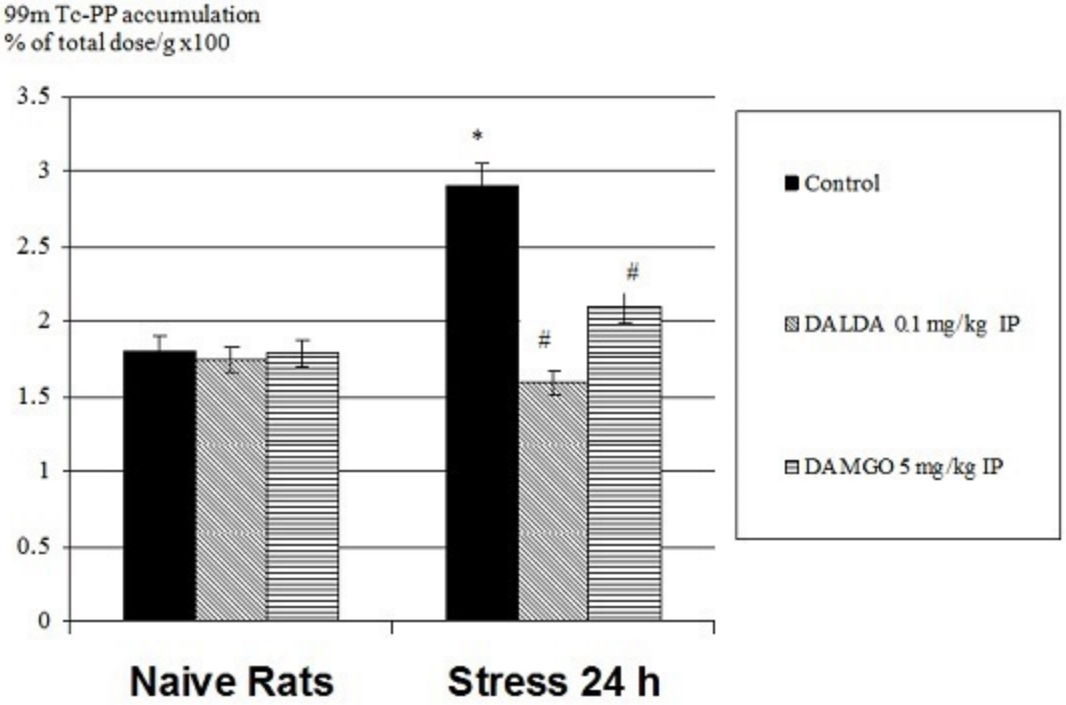
99m Tc-PP accumulation
% of total dose/g x100
3.5
3
2.5
2
1.5
1
0.5
0
*
#
#
Control
DALDA 0.1 mg/kg IP
DAMGO 5 mg/kg IP
Naive Rats
Stress 24 h

Fig 2a

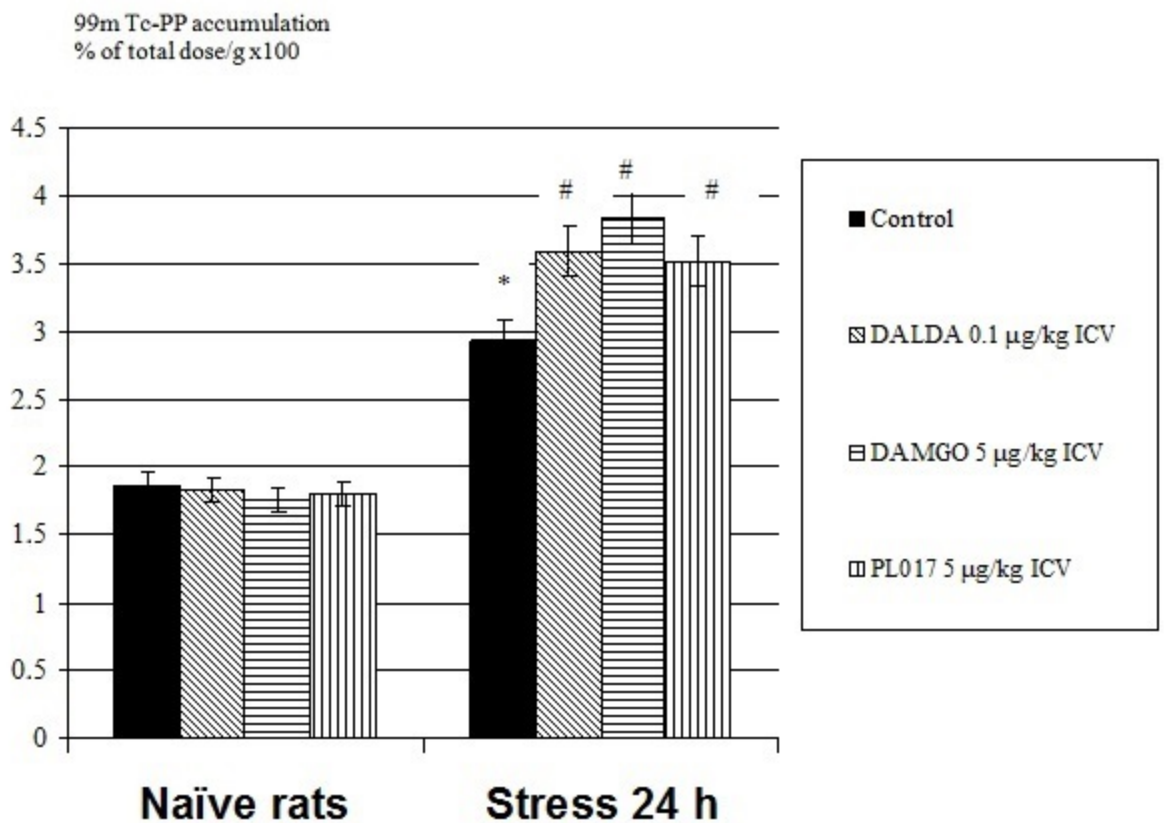
99m Tc-PP accumulation
% of total dose/g x100
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
#
#
#
Control
DALDA 0.1 µg/kg ICV
DAMGO 5 µg/kg ICV
PL017 5 µg/kg ICV
Naïve rats
Stress 24 h

Fig. 2b

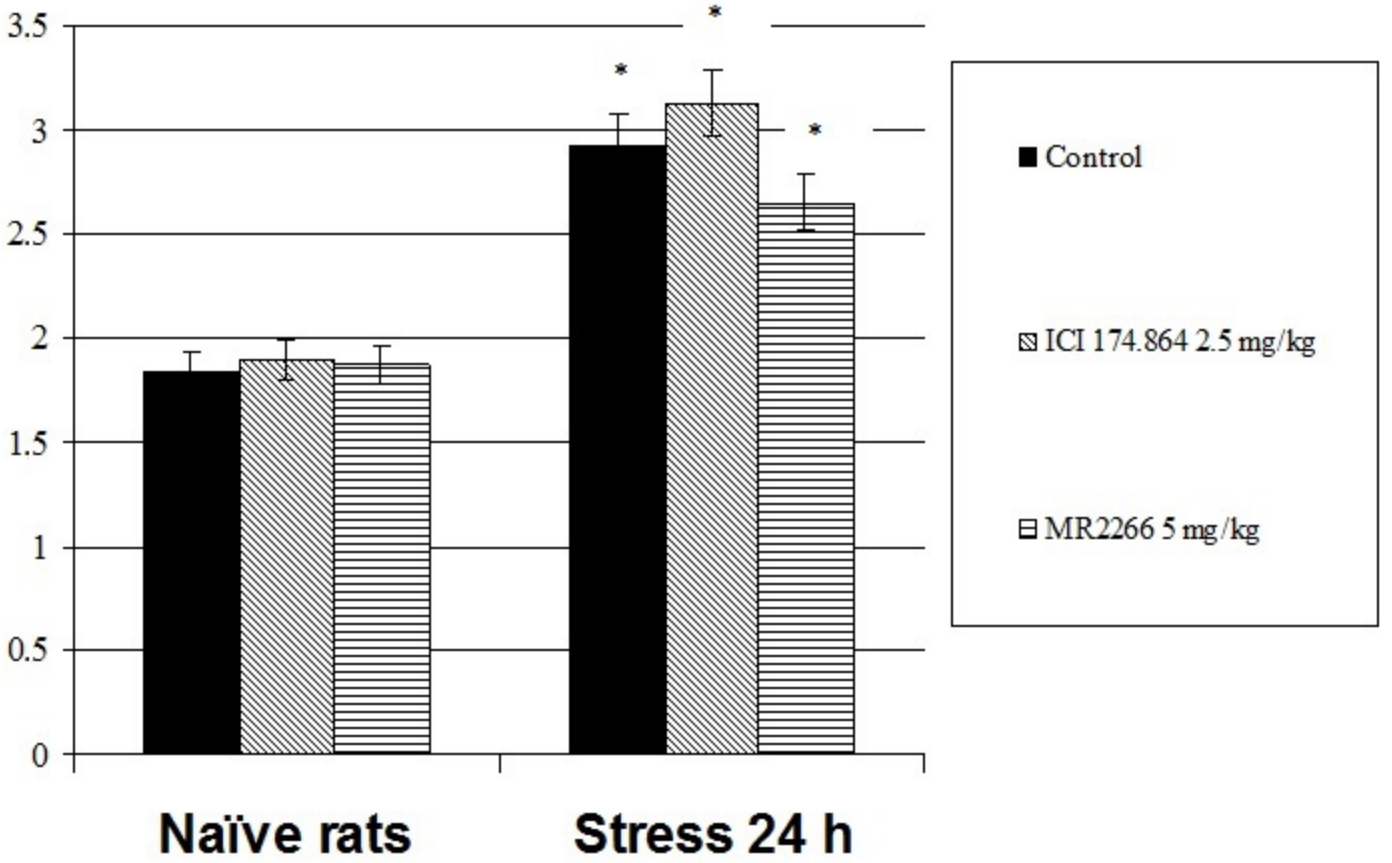
3.5
3
2.5
2
1.5
1
0.5
0
*
*
*
Control
ICI 174.864 2.5 mg/kg
MR2266 5 mg/kg
Naïve rats
Stress 24 h

Fig. 3

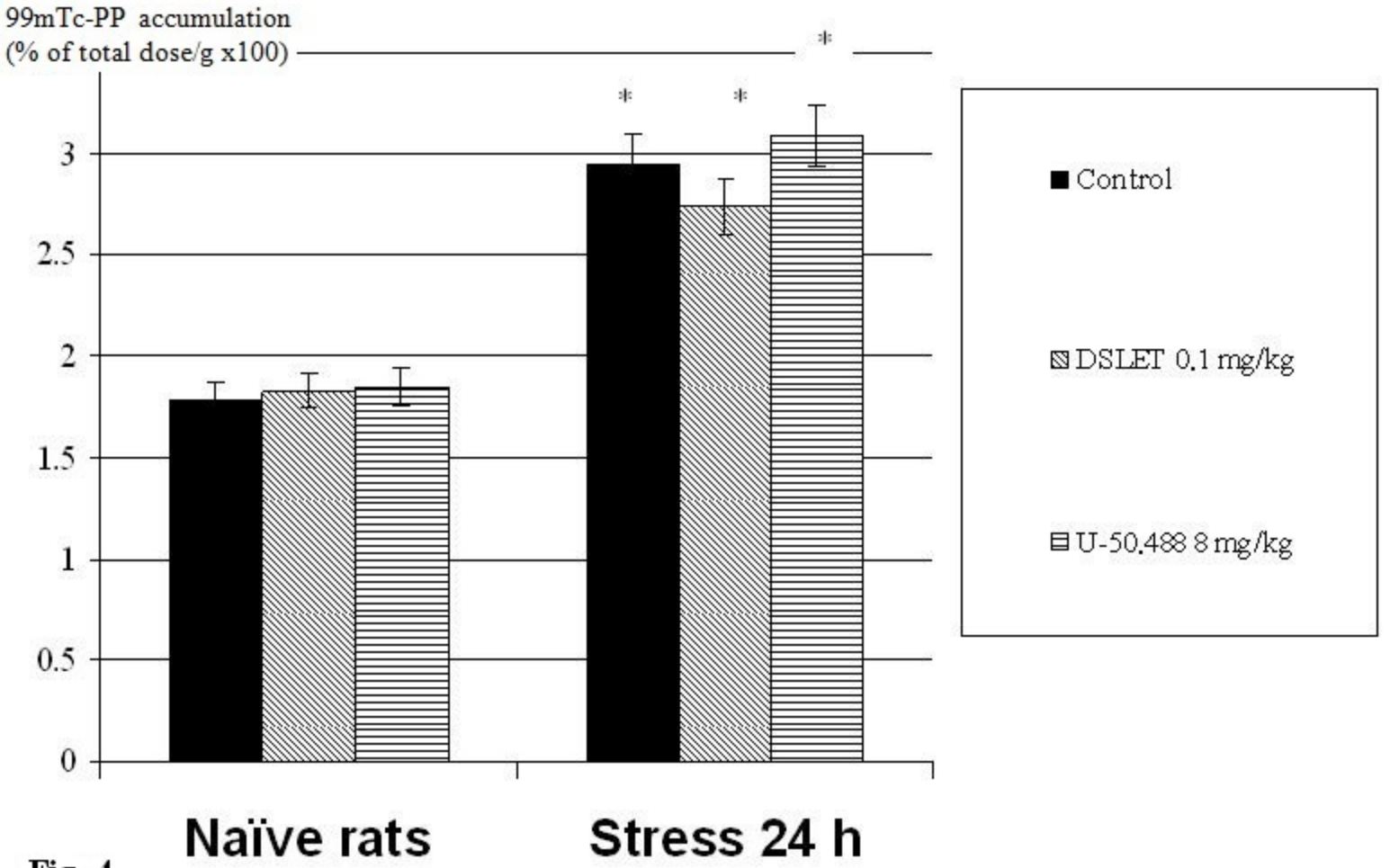
99mTc-PP accumulation
(% of total dose/g x100)
3
2.5
2
1.5
1
0.5
0
Control
DSLET 0.1 mg/kg
U-50,488 8 mg/kg
Naïve rats
Stress 24 h

Fig. 4

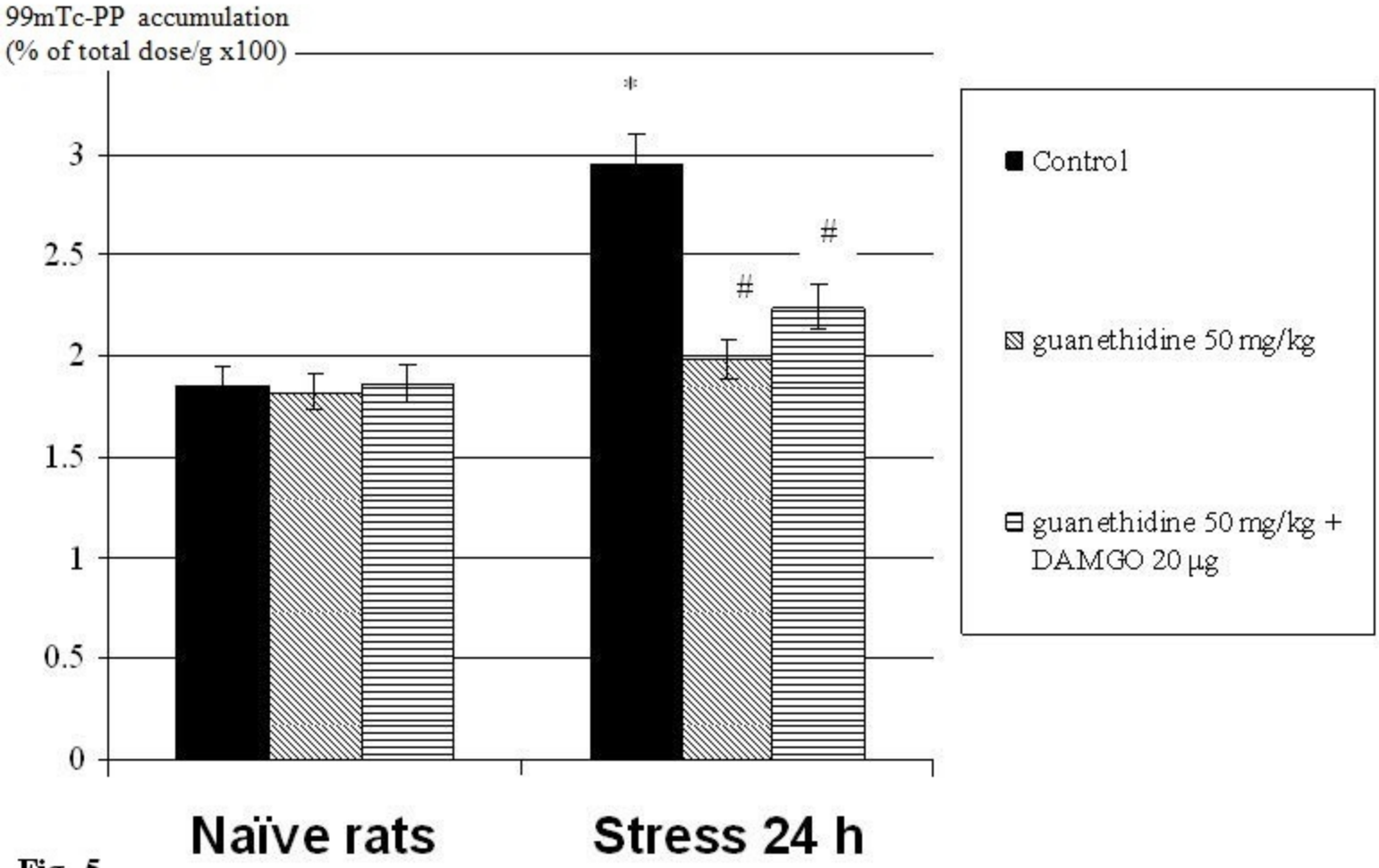
99mTc-PP accumulation
(% of total dose/g x100)
3
2.5
2
1.5
1
0.5
0
*
#
#
Control
guanethidine 50 mg/kg
guanethidine 50 mg/kg + DAMGO 20 µg
Naïve rats
Stress 24 h

Fig. 5