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Article in *Pharmacological reports: PR* · April 2014

Impact Factor: 1.93 · DOI: 10.1016/j.pharep.2013.09.003

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Original research article

Enhancement of antinociceptive effect of morphine by antidepressants in diabetic neuropathic pain model

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ARTICLE INFO

Article history:

Received 17 January 2013

Received in revised form 31 July 2013

Accepted 16 September 2013

Available online 4 March 2014

Keywords:

Diabetes

Streptozotocin-induced hyperalgesia

Amitriptyline

Morphine

Pain

ABSTRACT

Background: Recent studies have shown that **influence of antidepressants on analgesic action of opioids is heterogeneous**. The aim of this study was to investigate the effect of acute and repeated (21 days) antidepressant (amitriptyline, moclobemide and reboxetine) treatment on the antinociceptive action of morphine, an opioid agonist, in streptozotocin (STZ)-induced neuropathic pain model.

Methods: The studies were performed on the male Wistar rats. The changes in nociceptive thresholds were determined by using mechanical stimuli (the Randall–Selitto and the von Frey tests). Diabetes was induced by intramuscular administration of STZ.

Results: In this work we report that acute as well as repeated per os administration of antidepressants (amitriptyline, moclobemide and reboxetine) **significantly potentiated the antihyperalgesic effect of morphine in STZ-induced neuropathic pain model**.

Conclusion: **Combination therapy, such as classical antidepressants (amitriptyline, moclobemide) with opioids, or agents with noradrenaline reuptake inhibition and μ -opioid receptor activation could be a new target for research into treatment of painful diabetic neuropathy.**

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Introduction

It is well known that long-lasting hyperglycemia, associated with metabolic and cardiovascular alterations, often causes diabetic neuropathic pain (DNP) [44]. Typical symptoms of DNP include nagging pain followed by hyperalgesia and allodynia. Nowadays, management of DNP remains only a partially solved medical problem. The randomized controlled trials (RCTs) reported the efficacy of some opioids, such as oxycodone and tramadol in DNP [3]. Moreover, it was found that combination drugs treatment (morphine, oxycodone with gabapentin) had a better effect than each drug used alone in patients with DNP [9,12]. The relatively new opioid, tapentadol, with the dual mechanisms of action: μ -opioid agonism and noradrenergic reuptake inhibition, has demonstrated analgesic efficacy in preclinical and clinical studies with DNP. Therefore, the extended release form of tapentadol is approved by the United States Food and Drug Administration (FDA) for the

management of moderate to severe chronic pain, including diabetic peripheral neuropathic pain [14]. However, there is strong evidence of antidepressants effectiveness in DNP [41]. Tricyclic antidepressants (TCAs), such as amitriptyline, and selective serotonin and noradrenaline reuptake inhibitors (SSNRIs), such as duloxetine, are commonly recommended to treat neuropathic pain in diabetes patients [3]. In turn, efficacy of selective serotonin reuptake inhibitors (SSRIs) in neuropathic pain is contradictory. In addition, there is limited evidence of the effectiveness of monoamine oxidase inhibitors (MAOIs), such as moclobemide, and selective noradrenaline reuptake inhibitors (SNRIs), such as reboxetine [24].

It is believed that modulation of endogenous pain mechanisms through the serotonin and noradrenaline descending inhibitory pathways is a major mechanism of antinociceptive activity of antidepressants. Moreover, numerous studies have suggested activation of opioid endogenous system, blocking of noradrenoreceptors, muscarinic and histaminic receptors, blocking conduction in ions channels, activation of adenosine antinociceptive system, as well as peripheral modulation of inflammatory and immune parameters might be involved in the antinociceptive action of antidepressants [24,36].

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Numerous studies have shown that morphine, an opioid agonist, has incomplete analgesic activity in neuropathic pain therapy. However, it is commonly believed that adjuvant agents (e.g. antidepressants and anticonvulsants) augment opioid analgesia. There are many conflicting reports regarding the effect of combined prolonged administration of antidepressants with opioids. Some authors demonstrated increased analgesic effects of opioids (morphine) after prolonged therapy with antidepressants (imipramine, clomipramine) [11,37], whereas others showed decreased analgesic effects of opioids (morphine, fentanyl) after their prolonged administration with antidepressants (amitriptyline, imipramine, fluoxetine, moclobemide, reboxetine) [10,18].

Thus, the aim of this study was to investigate the effect of single and repeated administration of antidepressants (amitriptyline, moclobemide and reboxetine) on effectiveness of morphine in STZ-induced neuropathic pain model.

Materials and methods

Laboratory animals

This study was conducted according to the guidelines of the Ethical Committee for Experiments on Small Animals, Medical University of Warsaw, which approved the experimental protocols. Male Wistar rats (Laboratory of experimental animals, Medical University of Warsaw, Poland), weighing 250–350 g were housed in a room maintained at 20 ± 2 °C temperature and under 12–12 h light–dark cycles. Experimental groups consisted of six rats (2–3 rats were housed per cage). The total number of animals that we used in all experiments was 120. In the diabetic neuropathy model animals had a free access to food and water, except for a 16 h period before the first experimental session (STZ administration). Individual animals were used in one experiment, only.

Chemicals

Amitriptyline was obtained from ICN Polfa, Rzeszów, Poland; moclobemide was obtained from Anpharm, Poland; reboxetine was obtained from Pharmacia, Italy; morphine was obtained from Polfa Warszawa, Poland; STZ (*N*-[methylnitrosocarbonyl]- α -D-glucosamine) was purchased from Sigma Chemical Co., USA.

Equipment

Equipment included an analgesimeter (Ugo-Basile, Comerio, Italy), an Electronic von Frey anesthesiometer (Stoelting Co., Wood Dale, USA) and a glucometer (Accu-Check Active, Roche Diagnostics Corp.). The analgesimeter and the Electronic von Frey progressively increased pressure stimuli.

Streptozotocin-induced diabetes

Diabetes with accompanying painful neuropathy was induced by intramuscular (*im*) administration of STZ at a dose of 40 mg/kg body weight (b.w.), as described by Nakhoda and Wong [25] and Bujalska et al. [5].

Drugs administration

Streptozotocin was administered as described above.

Preparation of drugs

Morphine (MRF) was dissolved in 0.9% NaCl, whereas amitriptyline (AMI), moclobemide (MOC) and reboxetine (REB) were suspended in a 0.5% water solution of methylcellulose immediately prior to administration.

Administration of drugs

Antidepressants doses were selected on the basis of previous study [18] and screening test results. The MRF dose was selected as described previously [6]. AMI was administered orally (*po*) at 3 and 12 mg/kg, MOC at 5 and 10 mg/kg *po*, REB at 0.8 and 8 mg/kg *po*, whereas MRF was administered subcutaneously (*sc*) at a 5 mg/kg dose. The groups are presented below.

STZ + AMI – diabetic groups that on day 19 after STZ injection received acute administration of amitriptyline at doses 3 and 12 mg/kg; STZ + MOC – diabetic groups that on day 19 after STZ injection received acute administration of moclobemide at doses 5 and 10 mg/kg; STZ + REB – diabetic groups that on day 19 after STZ injection received acute administration of reboxetine at doses 0.8 and 8 mg/kg; STZ – diabetic group that on day 19 after STZ injection received acute administration of equivalent volume of 0.5% water solution of methylcellulose; control – healthy group that on day 19 after citrate buffered solution injection received acute administration of equivalent volume of 0.5% water solution of methylcellulose.

Time schedule

The antinociceptive action of MRF was determined after a premedication with a single antidepressant dose and after a 21-day antidepressant premedication in the diabetic (STZ)-induced neuropathy model.

Acute studies

The influence of single administration of antidepressants on the activity of MRF was investigated. Antidepressants were administered 1 h before MRF injection on day 19 of the experiment following an STZ injection. At this point, rats had developed hyperalgesia and we observed a similar reduction in their nociceptive thresholds in comparison to the values obtained before neuropathy. The control group received simultaneously MRF and a 0.5% water solution of methylcellulose. Nociceptive thresholds were determined 30, 60, 90, 120, 150, and 180 min after the MRF injection. The groups are presented below.

STZ + MRF – diabetic group that on day 19 after STZ injection received acute administration of morphine at dose 5 mg/kg with equivalent volume of 0.5% water solution of methylcellulose; STZ + AMI + MRF – diabetic group that on day 19 after STZ injection received co-administration of amitriptyline at dose 3 mg/kg with morphine at dose 5 mg/kg; STZ + MOC + MRF – diabetic group that on day 19 after STZ injection received co-administration of moclobemide at dose 5 mg/kg with morphine at dose 5 mg/kg; STZ + REB + MRF – diabetic group that on day 19 after STZ injection received co-administration of reboxetine at dose 0.8 mg/kg with morphine at dose 5 mg/kg; STZ – diabetic group that on day 19 after STZ injection received co-administration of equivalent volume of 0.9% NaCl with 0.5% water solution of methylcellulose; control – healthy group that on day 19 after citrate buffered solution injection received co-administration of equivalent volume of 0.9% NaCl with 0.5% water solution of methylcellulose.

Chronic studies

The effect of repeated administration of antidepressants on the analgesic action of MRF was studied. Antidepressants were administered daily from day 19 to day 39 of the experiment (21 days). This type of repeated treatment resembles the use of antidepressants in clinical practice and the same model of drug administration was used in previous publications [4,10,18]. On day 39, measurements of the nociceptive thresholds were conducted in the period from 30 min to 180 min after the MRF injection.

At the same time, the control group received a 0.5% water solution of methylcellulose once daily for 21 days and, on the last day of the experiment, simultaneously received MRF and a 0.5% water solution of methylcellulose. The groups are presented below.

STZ + MRF – diabetic group that on day 19 after STZ injection received a 0.5% water solution of methylcellulose once daily for 21 days and, on the last day of the experiment, simultaneously received morphine at dose 5 mg/kg with equivalent volume of 0.5% water solution of methylcellulose; STZ + 21 days AMI + MRF – diabetic group that on day 19 after STZ injection received amitriptyline at dose 3 mg/kg once daily for 21 days and, on the last day of the experiment, simultaneously received amitriptyline at dose 3 mg/kg with morphine at dose 5 mg/kg; STZ + 21 days MOC + MRF – diabetic group that on day 19 after STZ injection received moclobemide at dose 5 mg/kg once daily for 21 days and, on the last day of the experiment, simultaneously received moclobemide at dose 5 mg/kg with morphine at dose 5 mg/kg; STZ + 21 days REB + MRF – diabetic group that on day 19 after STZ injection received reboxetine at dose 0.8 mg/kg once daily for 21 days and, on the last day of the experiment, simultaneously received reboxetine at dose 0.8 mg/kg with morphine at dose 5 mg/kg; STZ – diabetic group that on day 19 after STZ injection received a 0.5% water solution of methylcellulose once daily for 21 days and, on the last day of the experiment, simultaneously received equivalent volume of 0.5% water solution of methylcellulose with 0.9% NaCl; control – healthy group that on day 19 after citrate buffered solution injection received a 0.5% water solution of methylcellulose once daily for 21 days and, on the last day of the experiment, simultaneously received equivalent volume of 0.5% water solution of methylcellulose with 0.9% NaCl.

Measurement of the nociceptive threshold

The Randall–Selitto test

Changes in nociceptive thresholds were determined using mechanical stimuli in the classic paw withdrawal test described by Randall and Selitto [35] with modification by Bujalska et al. [5].

The von Frey test

Changes in nociceptive thresholds were evaluated using an Electronic von Frey device. Rats were placed in individual plastic boxes with a metal mesh floor and allowed to adapt for 5 min. Gradually increased strength was applied to filaments through the mesh floor perpendicularly to the plantar surface of the hind paw. The intensity of mechanical stimuli was increased (range from 0.1 to 65 g) until the hind paw was withdrawn [15]. Threshold pressure values were recorded. The nociceptive threshold was obtained in triplicate and the mean was derived from further statistical calculations.

Statistical analysis

Changes in pain threshold were calculated as a percentage of baseline value according to the following formula:

$$\% \text{ of analgesia} = \left(\frac{B}{A} \times 100\% \right) - 100\%$$

where A indicates pressure (in g) at baseline (on day 1 of study, before STZ administration) and B indicates pressure (in g) recorded in the consecutive measurements that were performed after drugs administration. Percentage analgesia values received for individual

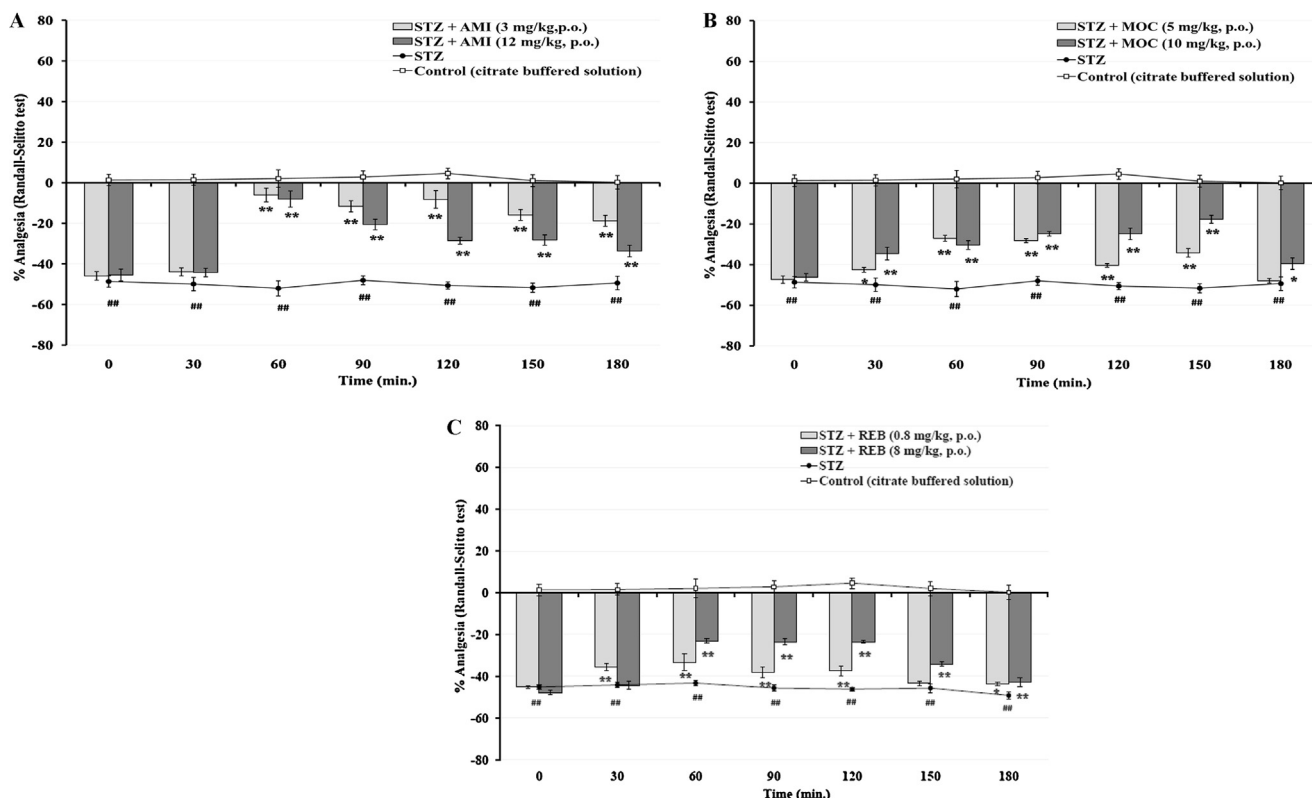


Fig. 1. Randall–Selitto test. Effect of single administration of amitriptyline (AMI) at doses of 3 and 12 mg/kg po (A), moclobemide (MOC) at dose of 5 and 10 mg/kg po (B), reboxetine (REB) at dose of 0.8 and 8 mg/kg po on the pain threshold to mechanical stimuli on day 19 of study. Values are shown as mean ± SEM. Control, diabetic rats: n = 6. AMI or MOC or REB vs. STZ *p < 0.05, **p < 0.01. STZ vs. control ##p < 0.01.

animals were used to calculate averages in individual experimental groups and for statistical analysis.

Results were expressed as mean ± standard error of the mean (SEM). The statistical significance of differences between the groups was evaluated by two-way analysis of variance (ANOVA) with replicate measurements, followed by the Fisher *post hoc* test; $p < 0.05$ and $p < 0.01$ were accepted as statistically significant. All statistical calculations were performed using Statistica, version 9.

Results

Development of mechanical hyperalgesia in streptozotocin-treated rats

The nociceptive threshold values reduced gradually after the STZ injection in the modified Randall–Selitto and von Frey test. Three days after injection STZ-treated rats developed mechanical hyperalgesia, peaking on day 17 in Randall–Selitto test and on day 16 in von Frey test, and remaining at a similar level until day 39 (data not shown).

The effect of different doses of antidepressants on hyperalgesia induced by STZ administration (dose selection)

The Randall–Selitto test was used for establishing the doses of amitriptyline, moclobemide and reboxetine to be applied in further experiments. Systemic administration of amitriptyline (3 and 12 mg/kg, *po*) produced a significant antihyperalgesic effect in the STZ-induced neuropathic pain model. The effect peaked at 60 min after 3 and 12 mg/kg b.w. drug administration and gradually decreased (Fig. 1A) (3 mg/kg: time effect: $F_{1,10} = 449.68$, $p < 0.01$; Fisher *post-hoc* test: STZ < AMI at $p < 0.01$ on minutes 60–180) (12 mg/kg: time effect: $F_{1,10} = 550.16$, $p < 0.01$; Fisher *post-hoc* test: STZ < AMI at $p < 0.01$ on minutes 60–180).

Administration of moclobemide (5 and 10 mg/kg b.w., *po*) significantly attenuated STZ hyperalgesia with maximal effect at 60 min after 5 mg/kg b.w. and at 90 min after 10 mg/kg b.w. after drug injection (Fig. 1B) (5 mg/kg: time effect: $F_{1,10} = 1170.83$, $p < 0.01$; Fisher *post-hoc* test: STZ < MOC at $p < 0.05$ on 30 min, at $p < 0.01$ on minutes 60–150) (10 mg/kg: time effect: $F_{1,10} = 832.12$, $p < 0.01$; Fisher *post-hoc* test: STZ < MOC at $p < 0.01$ on minutes 30–150 and $p < 0.05$ on 180 min).

Reboxetine administration (0.8 and 8 mg/kg b.w., *po*) significantly increased the nociceptive threshold in STZ rats. This effect was dose-dependent. The maximum effect was achieved 60 min after administration of reboxetine in both doses. The antihyperalgesic effect of the lower dose of reboxetine (0.8 mg/kg b.w., *po*) diminished at 150 min after drug administration. However, this effect of the higher reboxetine dose (8 mg/kg b.w., *po*) lasted up to the end of the experiment (Fig. 1C) (0.8 mg/kg: time effect: $F_{1,10} = 2491.67$, $p < 0.01$; Fisher *post-hoc* test: STZ < REB at $p < 0.01$ on minutes 30–120 and at $p < 0.05$ at 180 min) (8 mg/kg: time effect: $F_{1,10} = 4571.39$, $p < 0.01$; Fisher *post-hoc* test: STZ < REB at $p < 0.01$ on 60–180 min).

We decided to choose the 3 mg/kg b.w. dose of amitriptyline, the 5 mg/kg b.w. dose of moclobemide and the 0.8 mg/kg b.w. dose of reboxetine for further studies, because these were the first doses of those examined that decreased the STZ-induced hyperalgesia.

The effect of acute antidepressants treatment on the activity of morphine in STZ-induced pain model

Acute administration of morphine (5 mg/kg b.w., *sc*) induced only slight antihyperalgesic effect in diabetic neuropathic pain model in Randall–Selitto and von Frey tests. Acute administration of moclobemide and reboxetine significantly increased and

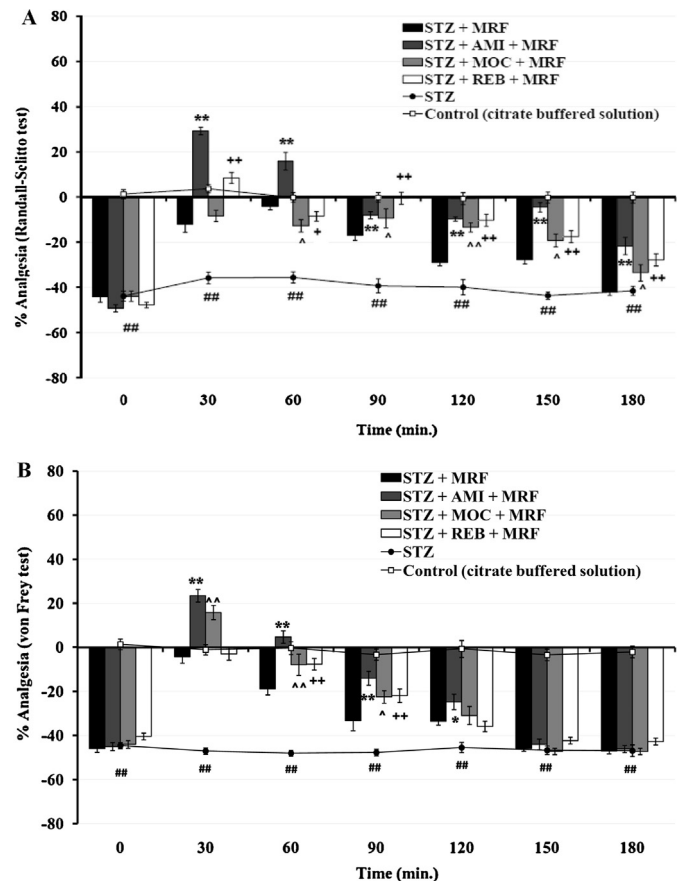


Fig. 2. (A) Randall–Selitto test, (B) von Frey test. The antinociceptive effect of co-administration of amitriptyline (AMI) at dose of 3 mg/kg, *po* or moclobemide (MOC) at dose of 5 mg/kg, *po*, or reboxetine (REB) at dose of 0.8 mg/kg, *po* with morphine (MRF) at dose of 5 mg/kg, *sc* in a streptozotocin (STZ) model of neuropathy in rats. Values are shown as the mean ± SEM. Control, diabetic rats: $n = 6$. AMI + MRF vs. MRF * $p < 0.05$, ** $p < 0.01$; MOC + MRF vs. MRF ^ $p < 0.05$, ^^ $p < 0.01$; REB + MRF vs. MRF + $p < 0.05$, ++ $p < 0.01$; STZ vs. control ## $p < 0.01$.

prolonged antihyperalgesic effect of morphine in both tests, while acute co-administration of amitriptyline with morphine produced strong analgesic effect at 30 and 60 min after the administration of these drugs (Fig. 2A and B). (A) Randall–Selitto test: (AMI + MRF: time effect: $F_{1,10} = 327.62$, $p < 0.01$; Fisher *post-hoc* test: MRF < AMI + MRF at $p < 0.01$ on minutes 30–180) (MOC + MRF: time effect: $F_{1,10} = 296.54$, $p < 0.01$; Fisher *post-hoc* test: MRF < MOC + MRF at $p < 0.05$ on 60, 90, 150 and 180 min, at $p < 0.01$ on 120 min) (REB + MRF: time effect: $F_{1,10} = 381.93$, $p < 0.01$; Fisher *post-hoc* test: MRF < REB + MRF at $p < 0.01$ on 30, 90, 120–180 min and at $p < 0.05$ on 60 min) and (B) von Frey test: (AMI + MRF: time effect: $F_{1,10} = 415.5$, $p < 0.01$; Fisher *post-hoc* test: MRF < AMI + MRF at $p < 0.01$ on minutes 30–90, at $p < 0.05$ on 120 min) (MOC + MRF: time effect: $F_{1,10} = 457.44$, $p < 0.01$; Fisher *post-hoc* test: MRF < MOC + MRF at $p < 0.01$ on minutes 30, 60 and at $p < 0.05$ on 90 min) (REB + MRF: time effect: $F_{1,10} = 733.011$, $p < 0.01$; Fisher *post-hoc* test: MRF < REB + MRF at $p < 0.01$ on 60 and 90 min).

The effect of repeated antidepressants treatment on the activity of morphine in STZ-induced pain model

Antidepressants (amitriptyline, moclobemide and reboxetine) administrated on 21 consecutive days *per se* considerably reduced STZ-produced hyperalgesia in Randall–Selitto and von Frey tests (data not shown). The antihyperalgesic effect of the chronic antidepressants treatment can be seen in Fig. 3A and B at time 0.

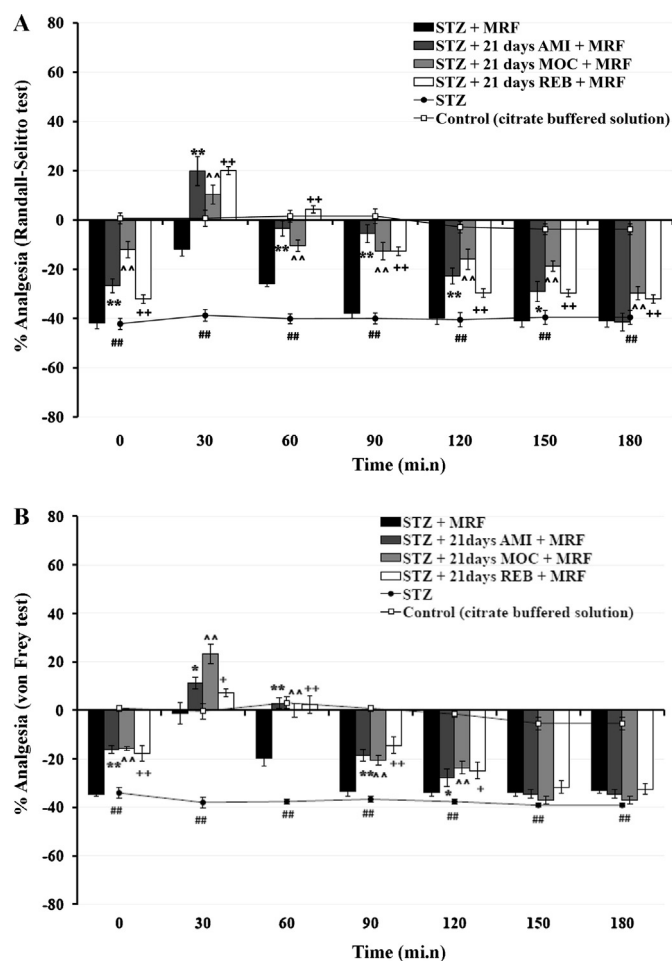


Fig. 3. (A) Randall–Selitto test, (B) von Frey test. The effect of chronic (21 days) administration of amitriptyline (AMI) at dose of 3 mg/kg, *po* or moclobemide (MOC) at dose of 5 mg/kg, *po*, or reboxetine (REB) at dose of 0.8 mg/kg, *po* on the antinociceptive activity of morphine (MRF) at dose of 5 mg/kg, *sc* in a streptozotocin (STZ) model of neuropathy in rats. Values are shown as mean \pm SEM. Control, diabetic rats: $n = 6$. 21 days AMI + MRF vs. MRF * $p < 0.05$, ** $p < 0.01$; 21 days MOC + MRF vs. MRF ^ $p < 0.05$, ^^ $p < 0.01$; 21 days REB + MRF vs. MRF + $p < 0.05$, ++ $p < 0.01$; STZ vs. control ## $p < 0.01$.

Repeated (21 days) administration of all antidepressants not only significantly potentiated and prolonged the antihyperalgesic effect of morphine, but also resulted in strong analgesic effect observed in 30 min after morphine administration in Randall–Selitto and von Frey tests. This effect gradually decreased until the end of the experimental period (180 min) in Randall–Selitto test or 120 min in von Frey test (Fig. 3A and B). (A) Randall–Selitto test: (21 days AMI + MRF: time effect: $F_{1,10} = 175.38$, $p < 0.01$; Fisher *post-hoc* test: MRF < 21 days AMI + MRF at $p < 0.01$ on minutes 0–120 and at $p < 0.05$ on 150 min) (21 days MOC + MRF: time effect: $F_{1,10} = 273.50$, $p < 0.01$; Fisher *post-hoc* test: MRF < 21 days MOC + MRF at $p < 0.01$ on 0–180 min) (21 days REB + MRF: time effect: $F_{1,10} = 530.96$, $p < 0.01$; Fisher *post-hoc* test: MRF < 21 days REB + MRF at $p < 0.01$ on minutes 0–180) and (B) von Frey test: (21 days AMI + MRF: time effect: $F_{1,10} = 352.66$, $p < 0.01$; Fisher *post-hoc* test: MRF < 21 days AMI + MRF at $p < 0.01$ on 0, 60 and 90 min, at $p < 0.05$ on 30 and 120 min) (21 days MOC + MRF: time effect: $F_{1,10} = 467.00$, $p < 0.01$; Fisher *post-hoc* test: MRF < 21 days MOC + MRF at $p < 0.01$ on 0–120 min) (21 days REB + MRF: time effect: $F_{1,10} = 352.66$, $p < 0.01$; Fisher *post-hoc* test: MRF < 21 days REB + MRF at $p < 0.01$ on 0, 60 and 90 min and at $p < 0.05$ on 30 and 120 min).

Discussion

The present study compares effects of three antidepressants (amitriptyline, moclobemide and reboxetine) with diverse action mechanisms after acute and long-term administration (21 days) on the hyperalgesic effect of morphine in STZ-induced neuropathic pain model. Single premedication of these antidepressants significantly increased and prolonged the antihyperalgesic effect of morphine; however, only premedication with amitriptyline reversed the streptozotocin hyperalgesia and activated antinociceptive action of morphine in Randall–Selitto and von Frey tests. Furthermore, long-term premedication of all antidepressants not only potentiated the antihyperalgesic effect of morphine, but also demonstrated remarkable antinociceptive effect in the rat model of neuropathic pain.

Singular acute injection of STZ (40 mg/kg *b.w.*, *im*) gradually decreased the nociceptive threshold, with peak values observed on day 17 in the modified Randall and Selitto test and on day 16 in the electronic von Frey test, and remained at a similar level until day 39. Nowadays, it was proven that both vascular and metabolic factors are involved in the development of diabetic neuropathy, which is typically accompanied by neuropathic pain and hyperalgesia. Long-lasting hyperglycemia leads to increase aldose reductase and sorbitol dehydrogenase activity, advanced glycation end products formation, oxidative-nitrosative stress, increase protein kinase C activity, as well as over-activation of poly(ADP-ribose) polymerase. All these changes not only contribute to damage of the peripheral neurons directly, but also lead to microvascular embolism, vasoconstriction that reduce the blood perfusion and impair the function of blood vessels supplying these neurons (*vasa nervorum*) [43].

The result obtained in this work demonstrated that single administration of amitriptyline (a tricyclic antidepressant), moclobemide (a MAO type A inhibitor) and reboxetine (a SNRI) suppressed STZ-induced hyperalgesia. These results confirm previous observations of Yamamoto et al. [46], who demonstrated improvement of the mechanical allodynia after *po* administration of amitriptyline at the same experimental model. In another work the authors revealed that moclobemide dose-dependently attenuated the mechanical hyperalgesia in a rat model of unilateral mononeuropathy [1]. Also, it was shown that reboxetine reversed thermal hyperalgesia in two models of neuropathic pain (chronic constriction injury – CCI and spinal nerve ligation – SNL), but at the higher doses than we used (3–10 mg/kg, *ip*) [32]. On the contrary, Juš et al. [18] showed that amitriptyline, moclobemide and reboxetine given alone were completely ineffective after acute administration in the Randall–Selitto model of acute pain. The causes of this discrepancy are unclear, but it could be due to different behavioral tests used. Differences in aforementioned observations need further studies to be explained.

In clinical practice, antidepressants are used for prolonged treatment to alleviate chronic pain. The current study showed that chronic administration of antidepressants (amitriptyline, moclobemide and reboxetine) for 21 days attenuated STZ-induced hyperalgesia (data not shown). These results confirm previous findings that have been demonstrated in both experimental and clinical studies [8,24].

Potential of the antinociceptive effect of opioids (tramadol, morphine, fentanyl) by the tricyclic antidepressants (doxepin, amitriptyline, imipramine) after acute [18,33,45], as well as sub-chronic [19,37] and chronic [11] pretreatment with the antidepressants have been described in the different pain models in rodents. However, Kellstein et al. [19] and Juš et al. [18] reported increasing antinociceptive effect of opioids (morphine, fentanyl) after single administration of TADs (clomipramine, desipramine, amitriptyline) and decreasing this effect after long-term adminis-

tration in acute pain models. Results obtained in this work are similar to described in paper by Gutiérrez et al. [11], who demonstrated that acute as well as chronic administration of imipramine increased the antinociceptive effect of morphine in arthritic rats. Overall, efficacy of the gold standard of analgetic antidepressants – amitriptyline in enhancement of antinociceptive effect of morphine in the neuropathic pain model could be explained by multiple mechanisms of analgesic action at central and peripheral level. Amitriptyline not only increases noradrenaline and serotonin concentration in the descending inhibitory pathways, but also directly and/or indirectly activates opioid endogenous system, blocks sodium as well as calcium channels, inhibits adenosine uptake, blocks *N*-methyl-D-aspartate receptors and increases GABA-B receptor function [24]. However, blocking of noradrenergic, histamine and muscarinic receptors by amitriptyline causes serious adverse effect and often this drug is less tolerated by patients as compared with other selective antidepressants.

This study for the first time shows that reboxetine, a selective noradrenaline reuptake inhibitor, potentiated the antihyperalgesic effect of morphine after acute administration and revealed the antinociceptive effect of morphine after 21 days pretreatment in STZ-induced neuropathic pain. These results are partially consistent with previous findings indicating that co-administration of opioid agonists (morphine, methadone, fentanyl) with noradrenaline reuptake inhibitors (maprotiline, desipramine, reboxetine) potentiated the antinociception of opioids in acute pain [18,21,33,38]. In addition, it was demonstrated that clonidine, an α 2-adrenoreceptor agonist, acts synergistically with morphine in the acute and neuropathic pain [28,29]. These data confirm participation of adrenergic system in nociceptive transmission. Moreover, it has been shown that chronic pain may contribute to phenotypic shift favoring noradrenergic mechanisms over opioid-mediated mechanisms [13]. For example, Shaqura et al. [39] reported that the number of μ -opioid receptor immunoreactive (MOR-IR) neurons as well as the density of MOR-IR nerve terminals in the spinal cord were significantly reduced in animals with 12 weeks of STZ-induced diabetes. Furthermore, these authors has also observed a significant decrease in membrane-bound MOR binding sites and MOR-stimulated G protein coupling in the dorsal horn of the spinal cord in diabetic rats as compared to controls. In the diabetic neuropathy model, morphine after intravenous administration induced antinociception, but at doses twice as high as those used in normal rats [7]. On the other hand, Omiya et al. [27] demonstrated an increased in the density of α 2-adrenoreceptors in the spinal cord two weeks after STZ injection as well as stronger of the antinociceptive response to clonidine in diabetic than in non-diabetic mice. Therefore, agents with dual mechanisms of action consisting of both μ -opioid receptor activation and noradrenaline reuptake inhibition, such as tapentadol, will probably create a new approach in treatment of painful diabetic neuropathy in humans.

Development of tolerance to the analgesic effects of opioids is a major disadvantage in clinical pain management. Morphine tolerance mechanism is a complex process and it may involve changes in serotonergic and noradrenergic systems [20,31], activates *N*-methyl-D-aspartate (NMDA) receptors [23], enhances proinflammatory cytokines expression (tumor necrosis factor- α , interleukin (IL)-1 β and IL-6) in spinal cord [34], leads to downregulation of spinal glutamate transporter and increases concentration of excitatory amino acids [42]. Numerous studies provide evidence that combination of antidepressants (fenfluramine, amitriptyline, venlafaxine, fluoxetine, imipramine) with morphine increases the analgesic effect of morphine and attenuates tolerance [2,16,30,31,47]. A similar effect was reported by Singh et al. [40] and Joshi et al. [17] for sub-chronic (9 days) bupropion-morphine and fluoxetine-morphine co-administration.

The study Luger et al. [22] has also showed that sub-chronic (8 days) concomitant administration of a minimum effective dose of fluvoxamine with a low dose of sufentanil, a selective μ -opioid receptor agonist, by continuous intravenous infusion enhanced antinociception and attenuated development of tolerance in tail-flick test in rats. Moreover, Nayebi et al. [26] demonstrated that chronic (30 days) co-injection of fluoxetine with morphine increased the analgesic effect of morphine and delayed the development of tolerance to morphine analgesia in normal mice and mice with skin cancer. Basing on the previous publications we can conclude that 21 days concomitant administration of antidepressants (amitriptyline, moclobemide and reboxetine) with morphine may reduce the development of tolerance to morphine analgesia in diabetic neuropathic pain model. However, most of these studies were conducted in the acute model of pain. Therefore, further studies are needed to confirm these observations in the neuropathic pain models.

Conflict of interests

None declared.

Funding

The research and preparation of the article were not supported by any sponsor.

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