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

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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.


## 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*-[methylnitrosocarbamoyl]-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 administrated 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.