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Mechanisms of morphine–venlafaxine interactions in diabetic neuropathic pain model

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ABSTRACT

Background: we investigated the possible mechanisms involved in the interactions of venlafaxine (VFX), a selective serotonin and noradrenaline reuptake inhibitor, and morphine (MRF), an opioid receptor agonist, after acute and chronic VFX treatment in diabetic neuropathic pain model (DNPM).
Methods: The studies were performed on male 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 streptozotocin. In order to investigate the mechanism of interaction, animals were also pretreated with naloxone (NLX), a nonselective opioid antagonist, yohimbine (YOH), a nonselective α_2-adrenergic antagonist, and p-chloroamphetamine (PCA), a neurotoxin that destroys serotonergic neurons. The μ-opioid receptors' density was determined with the use of radioligand binding assay.
Results: VFX potentiated antinociceptive action of MRF after acute administration of VFX and this effect was decreased by pretreatment of NLX, YOH and PCA. On the contrary, VFX administered for 21 days prior to MRF significantly decreased the analgesic action of MRF; this effect was augmented only after YOH pretreatment. Also, 21-days administration of VFX caused decreasing tendency in the number of μ-opioid receptors in the brain stem.
Conclusions: The results of our study show that single administration of VFX potentiates antinociceptive action of morphine in DNPM. This effect is probably mediated by both, noradrenergic and serotonergic systems. On the other hand, 21-days administration of VFX significantly decreases analgesic action of MRF. Moreover, there is a possibility that VFX acts as an antagonist of *N*-methyl-D-aspartate receptors.

Introduction

Diabetic neuropathy pain (DNP) is one of the most common chronic complications of diabetes. DNP affects 8–26% of diabetic patients, and it is even more frequently untreated (39%) [1]. Pharmacological treatment of DNP remains a challenging problem in modern medicine and consists of symptomatic therapies. The European Federation of Neurological Societies (EFNS) recommends in its latest guidelines to use antidepressants (duloxetine, tricyclic antidepressants and venlafaxine ER) and antiepileptics (pregabalin, gabapentin) for first line in diabetic neuropathic pain, while opioids are usually used for second or third line [2]. On current evidence, duloxetine is probably the most effective drug for diabetic neuropathy [3]. Among patients with diabetic peripheral neuropathic pain, continuous treatment with duloxetine was associated with a reduction in opioid use [4].

Venlafaxine (VFX), selective serotonin–noradrenaline reuptake inhibitor, is effective in treatment of painful diabetic neuropathy and is believed to improve the patients' quality of life [5]. The main mechanisms of analgesic action of VFX include an increase of the amount of noradrenaline and serotonin in the descending inhibitory pathways at supraspinal and spinal levels [6], as well as blocking of sodium channels and activating of μ- and δ-opioid receptors [7].

Morphine (MRF) is a strong opioid, which has unsatisfactory effect in painful diabetic neuropathy [5]. Hence, it is recommended

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in clinical practice to combine opioids and adjuvant drugs, such as antidepressants (AD) and antiepileptics [2].

In our previous study we have reported that VFX modified analgesic effect of MRF depended on the mode of the administration (single or long-term) in streptozotocin (STZ)-induced neuropathy pain model [8]. However, the mechanisms of these interactions are still unknown. Earlier studies demonstrated that interactions between MRF and antidepressants were mediated by serotoninergic, noradrenergic and opioidergic systems. Pettersen et al. [9] showed that, a nonselective α_2-adrenergic antagonist yohimbine (YOH) and, a nonselective opioid antagonist, naloxone (NLX) reversed the synergistic interaction between MRF and maprotiline, selective noradrenaline reuptake inhibitor. Sounvoravong et al. [10] revealed that ketanserine, a serotonin 2A receptor antagonist, and NLX blocked the combined antinociceptive effect of fluoxetine, a selective serotonin reuptake inhibitor, and MRF in neuropathic pain model.

The aim of this study was to investigate the mechanisms of the antihyperalgesic efficacy of VFX–MRF interactions after single and repeated administration of VFX using NLX, YOH and p-chloroamphetamine (PCA, neurotoxin destroying serotonergic neurons) in the STZ-induced neuropathy pain model. Additionally, we performed the radioligand binding assay to determine whether the 21-days administration of VFX altered the density of μ-opioid receptors in the cerebral cortex and brain stem.

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 (250–350 g) were housed in a room maintained at a temperature of 20 ± 2 °C, under a 12–12 h light–dark cycle. The experimental groups consisted of six rats. Animals had a free access to food and water, except for a 16 h period before the first experimental session (STZ administration) in the diabetic neuropathic pain model. Individual animals were used in one experiment only.

Chemicals

VFX was obtained from Pliva Hrvatska, Croatia; MRF was obtained from Polfa Warszawa, Poland; streptozotocin (*N*-[methylnitrosocarbamoyl]-α-D-glucosamine), yohimbine and p-chloroamphetamine were purchased from Sigma Chemical Co., USA; naloxone was purchased from Tocris biochemical, USA.

Equipment

Equipment included an analgesimeter (type 7200, Ugo-Basile, Comerio, Italy), an electronic von Frey anesthesiometer (Stoelting Co., Wood Dale, USA) and a blood glucometer (Accu-Check Active, Roche Diagnostics Corp.).

Animal models of neuropathic pain

Diabetes with accompanying painful neuropathy was induced by single intramuscular (*im*) administration of STZ at a dose of 40 mg/kg body weight (b.w.), as described by Nakhoda and Wong [11] and Bujalska et al. [12]. Starting on day 3 (72 h after STZ administration), glucose levels were determined using a glucometer. Blood samples were drawn from the tail vein. Permanent hyperglycemia was detected (≥400 mg/dl) in all STZ-treated rats. In vehicle-treated animals, glucose levels reached about 90 mg/dl and remained stable during the entire observation period. STZ-induced hyperglycemia was accompanied by a gradual decrease in body weight, an increase in food consumption, and a considerable increase in water intake.

Drugs preparation and administration

STZ was administered as described above.

Preparation of drugs. MRF and PCA were dissolved in 0.9% NaCl, NLX and YOH were dissolved in distilled water, whereas VFX was suspended in a 0.5% water methylcellulose (MC) solution immediately prior to administration.

Administration of drugs. VFX was administered per os (*po*) at 10 mg/kg dose [13], whereas MRF was administered subcutaneously (*sc*) at a 5 mg/kg dose [14]. Naloxone (1 mg/kg) and yohimbine (4 mg/kg) were injected intraperitoneally (*ip*), p-chloroamphetamine (10 mg/kg) was given *sc*.

Time schedule

The day of STZ injection was the first day of the experiment. The mechanisms of MRF–VFX interactions after single and repeated (21 days) administration of VFX and single MRF injection were determined after pretreatment with given in separate experiments NLX, YOH and PCA in the diabetic (STZ)-induced neuropathy model. Antidepressant drugs exert their co-analgesic effects usually already after the first dose. However, on account of the possible complex receptor-dependent effects (*e.g.* changes in the expression or/and sensitivity receptors), the shifts in action profile of the antidepressants after its prolonged administration should also be taken into account. Our previous observations and the works by other authors showed that potential changes in co-analgesic action should be visible within 3 weeks of administration. Type of repeated treatment used in this study resembles the co-analgetic use of antidepressants in clinical practice and the same model of drug administration was used in previous publications [15–17].

Study consisted of two main parts. In the first one, only the effect of administration VFX and MRF was studied. In the second one, the real interaction mechanism was examined. In the first part of the study, VFX was administrated once on day 19 (acute study) or once daily for 21 days (from day 19 to day 39 after STZ administration) in chronic study. MRF was given in single injection simultaneously with VFX on day 19 (acute VFX administration study) or on day 39 (repeated VFX administration study) of experiment. The choice of administration days (19 and 39) was caused by the fact, that threshold decrease reached its plateau phase on day 17 in R–S test and on day 16 in vF test, and remained approximately stable until day 39. In this part of acute and repeated study there were three control groups – animals received (**1**) citrate buffer in a single injection (instead of STZ) and then 0.5% MC (instead of VFX; single dose on day 19 [acute study] or once daily for 21 days [19–39], chronic study) with single dose 0.9% NaCl (instead of MRF) on day 19 (acute study) or 39 (chronic study) or (**2**) STZ and single dose of MRF (day 19 or 39) and 0.5% MC solution instead of VFX (days as control 1), as well as (**3**) STZ in single injection and then 0.5% MC (instead of VFX; days as control 1) with single dose 0.9% NaCl (instead of MRF; days as control 1). The nociceptive thresholds in this part were determined on day 19 (acute VFX) or on day 39 (repeated VFX) of study, 30 min before VFX/MRF administration (baseline value) and at 30, 60, 90, 120, 150 and 180 min after MRF–VFX injection. Timing of thresholds measurements was taken from earlier an work by Juś et al. [17]. For controls, baseline thresholds values were determined 30 min before administration of (controls 1 and 3) 0.9% NaCl with 0.5% MC and (control 2) MRF in 0.9% NaCl + 0.5% MC; next