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Original research article

Analgesic effects of antidepressants alone and after their local co-administration with morphine in a rat model of neuropathic pain

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ABSTRACT

Background: The therapy of neuropathic pain may include the use of co-analgesics, such as antidepressants, however, their desired analgesic effect is associated with significant side effects. An alternative approach to this is their local administration which has been proposed, but there is little data regarding their local co-administration with morphine and the nature of the interaction between morphine and either doxepin or venlafaxine, two antidepressant drugs that have been recently used in neuropathic pain therapies.
Methods: This study was performed on rats after chronic constriction injury (CCI) to the sciatic nerve. The von Frey and Hargreaves' tests were used to assess mechanical allodynia and thermal hyperalgesia, respectively, after intraplantar (*ipl*) or subcutaneous (*sc*) administration of amitriptyline, doxepin, or venlafaxine, or their *ipl* co-administration with morphine on day 12–16 after injury.
Results: The *ipl* administration of amitriptyline (3, 15 mg), doxepin (1, 5, 10, 15 mg), or venlafaxine (2, 7 mg) was effective in antagonizing CCI-induced allodynia. Their *sc* injection at a site distal to the injured side, did not induce alterations in pain thresholds, which supports the local mode of action. Of the three antidepressants used in this study, only *ipl* co-administration of amitriptyline with morphine significantly enhanced its effect in contrast to doxepin and venlafaxine, both of which weakened the analgesic effect of morphine.
Conclusions: In summary, the results suggest that when amitriptyline (but not doxepin or venlafaxine) is locally co-administered with morphine the effectiveness under neuropathic pain is enhanced, although additional studies are necessary to explain differential mechanisms of interaction of antidepressant drugs with morphine after local administration.

Introduction

Neuropathic pain is defined as pain arising as a direct consequence of a lesion or disease affecting the somatosensory system [1]. The most frequent etiologic factors of neuropathic pain are trauma (including post-surgery scars), metabolic disturbances (e.g., diabetes mellitus) and ischemia. The pathological mechanism of neuropathic pain differs significantly from that of inflammatory pain; studies have shown that the changes in spinal gene expression under neuropathy and inflammations are different [2]. Thus, the response to antinociceptive drugs, especially opioids, is not the same. It has generally been accepted that neuropathic pain is somewhat resistant to morphine administration in clinical studies [3,4], and the reduced ability of morphine to attenuate allodynia and hyperalgesia in experimental models of neuropathic pain has been demonstrated [5–8]. For these reasons, it is a common clinical practice to use analgesic drug combinations. The primary approach to treat neuropathic pain is the use of coanalgesics such as tricyclic antidepressants (TCA); however, their systemic application is associated with significant side effects, which can hinder the desired analgesic effect. An alternative approach to this is the topical administration of analgesics. Currently, the possibility of the topical

Abbreviations: CCI, chronic constriction injury; MOR, morphine; AMT, amitriptyline; DOX, doxepin; VFX, venlafaxine; *ipl*, intraplantar; *sc*, subcutaneous.

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use of these drugs that have local analgesic effects has been proposed [9–14], but there is little data regarding antidepressant topical coadministration with morphine and the nature of the interaction between morphine and either doxepin or venlafaxine, two antidepressant drugs that have been recently used in neuropathic pain therapies.

Tricyclic antidepressants are the most studied group of antidepressants for the treatment of neuropathic pain. These drugs diminish the transmission of nociceptive information from the site of injury by inhibiting the reuptake of serotonin and norepinephrine at the synapse, which leads to prolonged activation of the antinociceptive descending pathways. Interestingly, pain relief appears to be independent of the primary antidepressant effects of these drugs. The effects of coadministration of opioids and antidepressants in different chronic pain models were shown by some authors. The synergistic action of morphine with amitriptyline or doxepine in preclinical and clinical study was demonstrated by some groups [15–18]. Wrzosek et al. [19] has shown that the combined administration of tramadol with doxepin was more effective than tramadol and venlafaxine, which provides valuable information regarding clinical practices and rationalizing the administration of different drug combinations. It should be noted that venlafaxine is a structurally novel antidepressant that inhibits reuptake of 5-hydroxytryptamine and noradrenaline, but unlike older generation of antidepressants, it has few side effects and has become increasingly popular in the treatment of pain [20,21]. Considering the possible local analgesic effects of antidepressants, which may be crucial when they are used in combination with morphine, we evaluated whether the local use of antidepressants (amitriptyline, doxepin and venlafaxine) is effective in the chronic constriction injury model of neuropathic pain in rats and if local coadministration of these antidepressants with morphine could influence its analgesic effect under neuropathic pain conditions.

Materials and methods

Animals

Male Wistar rats (250–350 g) were obtained from Charles River (Hamburg, Germany) and housed in cages lined with sawdust under a standard 12/12 h light/dark cycle (lights on at 08:00 h) with food and water provided ad libitum. All experiments were performed according to the recommendations of IASP [22] and the NIH Guide for the Care and Use of Laboratory Animals and were approved by the local Bioethics Committee (Kraków, Poland).

Surgical preparations

Chronic constriction injury (CCI) was generated as previously described by Bennett and Xie [23]. Four ligatures were tied around the sciatic nerve under sodium pentobarbital anesthesia (60 mg/kg; *ip*). The biceps femoris and the gluteus superficial were separated, and the right sciatic nerve was exposed. The ligatures (4/0 silk) were tied loosely around the nerve distal to the sciatic notch at 1 mm distances until they elicited a brief twitch in the respective hind limb. After surgery, all of the animals (100%) developed long-lasting neuropathic pain symptoms, including tactile allodynia and thermal hyperalgesia, which was demonstrated in our previous papers [6,24–26]. The difference in response of the ipsilateral paw to von Frey filaments approximately 12-16 days after nerve injury in the CCI rats compared to the control rats was: 0.8 g ± 0.03 *vs.* 26 g ± 0.01, respectively, whereas the differences in the paw withdrawal test were 5.6 s ± 0.4 *vs.* 8 s ± 0.6, respectively. The behavioral tests were conducted at 12–16 days after injury when neuropathic pain symptoms, like allodynia and hyperalgesia have been constant and persistent.

Drug administration

Amitriptyline hydrochloride [3-(10,11-dihydro-5H-dibenzo[a,d]cyclopenten-5-ylidine) propyldimethyloamine], doxepin hydrochloride [11-(3-dimethylaminopropylidene)-6,11-dihydrodibenz [b,e] oxepine hydrochloride] and venlafaxine hydrochloride [(+/-)-1-[2-(dimethylamino)-1-(4-methoxyphenyl) ethyl] cyclohexanol hydrochloride] were obtained from Sigma–Aldrich, (Poznań, Poland). Morphine hydrochloride was obtained from Polfa (Kutno, Poland). Drugs were administered to the rats by one of two methods: (1) intraplantar (*ipl*) injection of a volume of 20 μl per each animal to the ipsilateral hind limb sole, or (2) subcutaneous (*sc*) injection of a volume of 4 ml/kg body weight into the skin fold on the neck.

The range of doses was chosen according to our previous study [19] after *ip* injection and according to preliminary experiments with *ipl* and *sc* antidepressant administration. Most of the drugs (amitriptyline hydrochloride, doxepin hydrochloride and morphine hydrochloride) were dissolved in the *aqua pro injection*, but venlafaxine hydrochloride was dissolved in 12% dimethyl sulfoxide (DMSO). Control animals were injected *ipl* with the same volume of the respective vehicle. The dosages of the drugs for *sc* injections were calculated according to the body weight of each animal and were tested in accordance with the same schedule as described below. After completion of the experiment, the animals were euthanized by CO_2 asphyxiation.

Behavioral tests

Behavioral tests were conducted on days 12-16 after injury. Eight animals per an experimental group were used, with each animal used for one treatment only. In control groups, the number of animals was higher (up to 16) because the control group was included in each experiment and the results were pooled. In behavioral experiments, the control group comprised vehicle-treated (*ipl* or *sc*) CCI animals.

Tactile allodynia (von Frey test)

Allodynia was measured before and 15, 30 and 60 min after *ipl* drug administration in the CCI rats. A set of calibrated nylon von Frey filaments (Stoelting, Chicago, IL, USA) was used. Animals were placed in plastic cages with wire net floor 5 min prior to experimentation. Increasing filament strengths were applied sequentially to the midplantar surface of the hind paw. The intensity of mechanical stimulation was increased from 0.2 to 26 g in a graded manner using successive filaments with greater pressure until the hind paw was withdrawn as previously described [6,19,27]. To determine tactile allodynia in rats, the strength of the von Frey stimuli ranged from 0.5 to 26 g. The ipsilateral paw was tested in animals in 4 cages twice over 3 min intervals using von Frey filaments, and the mean values were calculated.

Paw withdrawal test–Hargreaves' test

Thermal hyperalgesia was measured on days 12-16 after induction of CCI at times before and 20, 45 and 75 min after *ipl* drug administration in the CCI rats. The pain threshold to high temperatures was tested using an Analgesia Meter (Landing, NJ). CCI rats were placed into 4 individual plastic cages with a glass floor 5 min prior to experimentation. A noxious thermal stimulus was focused through the glass onto the plantar surface of a hind paw until the animal lifted the paw away [6,28]. The cut-off latency was 20 s. The ipsilateral paws of the animals in 4 cages were tested twice over 3 min intervals, and the mean values were calculated.