Neuroscience Letters 568 (2014) 6–11

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

The analgesia effect of duloxetine on post-operative pain via intrathecal or intraperitoneal administration

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HIGHLIGHTS

- We modeled a post-operative pain.
- Duloxetine delivery produced an anti-hyperalgesic effect in postoperative pain model.
- The effect of duloxetine was partly attenuated by antagonists for 5-HT2A or α2-noradrenergic receptors.
- 5-HT and NA concentrations at the spinal dorsal horn were increased after duloxetine injection.

ARTICLE INFO

Article history:
Received 9 January 2014
Received in revised form 11 March 2014
Accepted 19 March 2014

Keywords:
Duloxetine
Post-operative pain
Serotonin-norepinephrine reuptake inhibitors
Mechanical hypersensitivity

ABSTRACT

One promising strategy to prevent the chronicity of post-operative pain (POP) is to attenuate acute POP during the early phase by efficacious medications with fewer side effects. Duloxetine, one of the serotonin (5-HT)-norepinephrine (NE) reuptake inhibitors (SNRI), is used to treat a wide range of acute and chronic pain. However, its effect on POP has not been investigated. In the present study, we investigated the anti-hypersensitivity effect of duloxetine using a rat model of POP. The possible involvement of spinal 5-HT_{2A} and α2-noradrenergic receptors were also evaluated by using antagonists for 5-HT_{2A} (ketanserin) or α2-noradrenergic receptors (idazoxan). Finally, with the method of in vivo microdialysis, the increase in spinal NA and 5-HT levels after intraperitoneal (i.p.) delivery of duloxetine were investigated. The results showed that intrathecal (i.t.) or i.p. delivery of duloxetine produced an anti-hyperalgesic effect in a dose-dependent manner. The anti-hypersensitivity effect of duloxetine was partly attenuated by pretreatment with ketanserin or idazoxane. Microdialysis study revealed that 5-HT and NA concentrations at the spinal dorsal horn were increased, peaking at 30 min after i.p. injection of 20 mg/kg duloxetine. These findings indicate that duloxetine inhibits POP by increasing spinal NA and 5-HT levels and activating spinal 5-HT_{2A} or α2-noradrenergic receptors.

1. Introduction

Post-operative pain (POP) is the commonly experienced pain following surgeries [22] and its chronic form contributes to the reduced quality of life in both adult [3,6] and adolescent [9] patients. Logistic regression revealed that the acute POP intensity is one independent predictor for persistent POP [25]. Therefore, one promising strategy to prevent the chronicity of POP is to attenuate acute POP during the early phase after operation.

Opioids remain the most frequently prescribed analgesics to manage the moderate to severe acute POP [7]. However, regardless of their administration methods, all opioids produce undesired side effects, including nausea and vomiting, pruritus, constipation, urinary retention, hyperalgesia, dependence and tolerance. On the other hand, physicians are often reluctant to prescribe opioids because of these side effects [13]. Indeed, ameliorating or eliminating these side effects is increasingly one of the most important challenges for pain management. Thus, research efforts

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http://dx.doi.org/10.1016/j.neulet.2014.03.046
0304-3940/

are put on exploring more effective strategies with fewer side effects.

Duloxetine, one of the serotonin (5-HT)-norepinephrine (NE) reuptake inhibitors (SNRI), inhibits the transporters for 5-HT and NE, thereby increasing local levels of these neurotransmitters [8,14] and promoting persistence of their actions. 5-HT exerts its analgesic effect through binding to a range of receptors [12], including the 5-HT_{2A} receptor [1]. Indeed, neuropathic pain is reported to be alleviated by activating spinal 5-HT_{2A} receptors [11]. Furthermore, duloxetine demonstrate synergistic analgesia when combined with other analgesics in our previous study [24]. However, it is not clear whether duloxetine is effective in treating acute POP and what the underlying mechanism is, whether the spinal cord is involved in the duloxetine analgesia on acute POP.

Thus we raised the hypothesis that duloxetine, either intrathecally (i.t.) or intraperitoneally (i.p.) administered, attenuates the incision induced POP during the early time window after operation via increasing the spinal 5-HT and NE levels. Using a rat hindpaw incision induced POP model [4], we compared the dose-analgesia effect of these two delivery routes and explored the underlying spinal mechanisms by blocking 5-HT_{2A} or α2-noradrenergic receptor with ketanserin or idazoxan, respectively. Furthermore, the in vivo alterations of spinal 5-HT and NA levels during i.p. delivery of duloxetine were further investigated with microdialysis technique.

2. Methods

2.1. Animal and drugs

Male *Sprague-Dawley* rats (250 g, about 4-month old) bred in the animal center of the Fourth Military Medical University (FMMU, Xi'an, China) were received 1 week before experiment and housed in a temperature-controlled environment on a 12-h light/dark cycle with free access to food and water ad libitum. All experimental procedures received prior approval from the Animal Use and Care Committee for Research and Education of FMMU (Xi'an, China) (permit number: 10302), and the ethical guidelines to investigate experimental pain in conscious animals was followed. Duloxetine (Eli Lilly Company, USA) was purchased and freshly dissolved in sterile saline, filtered before use. 5-HT_{2A} receptor antagonist ketanserin and α-noradrenergic receptor antagonist idazoxan were purchased from Sigma.

2.2. Operation

i.t. catheter implantation was performed under 2% (w/v) sodium pentobarbital anesthesia (40 mg/kg, i.p.) according to our previous studies. Briefly, a midline incision (3 cm) was cut on back of the rat at the level of the thoracic vertebrae. A pre-measured PE-10 tube was passed caudally from T_8 to L_3 level of the spinal cord, fixed at the back of rat's ears through subcutaneous tunnel, with 2 cm free end exposed in the upper thoracic region. Rats were allowed to recover for 3 days and only those judged as no neurological deficit were used for paw incision. Briefly, after sterile preparation and anesthetization, a 1-cm long incision was made in the plantar aspect of the right hindpaw by elevating and incising longitudinally the plantaris muscle.

2.3. Behavioral test

Before behavioral test, the animals were allowed to get used to the dimly illuminated (60 lx) experiment room for 30 min. The paw withdrawal threshold (PWT) to mechanical stimuli was tested using von Frey filaments (Stoelting, Kiel, WI, USA) during 8:00 to 12:00 in the dimly illuminated room. The stiffness of the von Frey filaments was 2, 4, 6, 8, 10, 15, and 26 g. Filaments were applied vertically to an area adjacent to the wound for 6 s, using just enough pressure to gently bend the filament. Acute withdrawal, biting, licking or shaking of the ipsilateral hind limb were considered to be positive signs of withdrawal. The behavioral testing was performed blindly with respect to the drug administration.

2.4. Microdialysis and high performance liquid chromatography (HPLC)

According to our previous study [5], microdialysis and HPLC were performed to measure NA and 5-HT levels in the spinal dorsal horn using rats with a paw incision (24 h after incision). A microdialysis probe and PE-10 tube were inserted into the lumbar intrathecal space from the L5 and L6 vertebrae. All rats were allowed to recover from implantation for 1 day. Only the animals that were neurologically normal after implantation surgery were used in the followed experiments. The microdialysis probe was connected to a syringe pump (CMA 402, Stockholm, Sweden), and the outlet cannula was connected to the microfraction collector (CMA 142, Stockholm, Sweden). The probe was perfused with artificial cerebrospinal fluid (ACSF) at a flow rate of 2 μL/min. After 120 min of constant perfusion, 2 consecutive samples were collected to determine basal NA and 5-HT concentrations in the dialysate. Saline (0.5 mL) or duloxetine (20 mg/kg) was i.p. administered through an indwelling catheter and dialysis samples were then collected every 15 min for 180 min and frozen at −80 °C. A Rainin A1 autosampler onto a Luna C18 column (250 mm × 4.6 mm) (Phenomenex, Torrance, CA) was used at a flow rate of 1 mL/min with a mobile phase consisting of 10 mM ammonium phosphate, pH 6.0, with 15% methanol. NA and 5-HT were determined using a Rainin Dynamax Model UV-D II absorbance detector at 254 nm.

2.5. Experiment plan

The PWTs to mechanical stimuli were measured before and at 3 days after i.t. implantation (24-h pre-incision operation). There was no significant difference between these two measurements, thus the PWTs measured at 24-h pre-operation were used as the pre-PWTs in the current study. The baseline PWTs (Base-PWT) were measured at 24-h after the paw incision and then followed by drug testing. Rats received i.p. (10, 20, 40 mg/kg, in a volume of 0.5 mL) or i.t. (10, 20, 40 μg/kg, in a volume of 5 μL) injection of duloxetine or the same volume of sterile saline (vehicle to dissolve duloxetine). Antagonist studies were performed to test whether the effect of duloxetine in the POP model is mediated through the spinal α2-adrenergic receptors (idazoxan) or 5-HT_{2A} receptor subunits (ketanserin). According to a previous study [20], 5 μL of saline, 30 μg of idazoxan or 20 μg of ketanserin was i.t. administered 15 min before duloxetine injection (either i.t. or i.p.) followed by a 10 μL saline injection to flush the catheter.

In all the behavioral experiment, PWTs were measured at 15-min interval for 180 or 90 min after i.p. or i.t. injection with duloxetine or vehicle, respectively. The cerebral spinal fluid (CSF) was collected via the i.t. tube at 15-min interval for 180 min after i.p. injection with duloxetine (20 mg/kg) or vehicle. These collected CSFs were used for microdialysis measurement of spinal 5-HT or NE. All the behavioral test and CSF collection were performed during 8:00 to 12:00 in the dimly illuminated room. For every group in the behavioral test or microdialysis analysis, six rats were used and rats for microdialysis analysis were naïve to behavioral test.

2.6. Statistics

The data were expressed as mean value ± standard error of the mean (SEM). When comparing the PWLs of incision animals with