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Antinociceptive effect of botulinum toxin type A on experimental abdominal pain

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ABSTRACT

Visceral pain, especially in the abdominal region, represents one of the most common types of pain. Its chronic form is usually very hard to treat by conventional analgesic agents and adjuvants. We investigated the antinociceptive effect of botulinum toxin type A (BTX-A) in male Wistar rats in two models of visceral pain: peritonitis induced by intraperitoneal injection of 1% acetic acid and colitis induced by intracolonic instillation of 0.1% capsaicin. Pain was measured as the number of abdominal writhes. Additionally, referred mechanical sensitivity in the ventral abdominal area was evaluated by von Frey test and the extent of spinal c-Fos expression was immunohistochemically examined. BTX-A significantly reduced the number of abdominal writhes in both models of visceral pain after intrathecal application in a dose of 2 U/kg. In the experimental colitis model, BTX-A (2 U/kg) reduced both referred mechanical allodynia and c-Fos expression in the dorsal horn of the spinal cord (S2/S3 segments). In contrast to intrathecal administration, BTX-A (2 U/kg) administered into the cisterna magna had no effect on pain suggesting that the primary site of its action is a spinal cord.

1. Introduction

Botulinum toxin type A (BTX-A) inhibits neurotransmitter release due to its endopeptic activity against SNAP-25 (SyNaptosomal Associated Protein of 25 kDa). This enables its therapeutic use in disorders characterized by overactive muscles, overactive exocrine glands and, most recently, non-muscular pain conditions (reviewed by Dressler (2013) and Matak and Lacković (2014)). Antinociceptive activity of BTX-A was demonstrated in various preclinical (reviewed by Pavone and Luvisetto (2010)) and clinical studies (reviewed by Jabbari and Machado (2011)) during the last decade. It was shown that a single injection reduces pain over a prolonged period of time (several months), which represents the unique feature of BTX-A among available analgesics.

Visceral pain, defined as pain arising from the internal organs of the body, is one of the most common forms of pain. In contrast to somatic pain, key features of visceral pain are diffuse localization typically referred to somatic sites (e.g. muscle and skin), stronger emotional and autonomic reactions, an unreliable association with pathology, and intense referred hyperalgesia (Ness, 1999; Sanoja et al., 2010). Despite of these differences, treatment has been the same as for somatic pain: based on non-steroidal anti-inflammatory drugs (NSAIDs), opioids and adjuvant analgesics like tricyclic antidepressants, antiepileptic drugs etc.

Unlike acute forms of visceral pain, chronic visceral pain might be refractory to the listed drugs because of their short duration of action, variable effectiveness, and unwanted side effects (Davis, 2012). Thus, there is a need for new therapeutic options with better effectiveness, longer duration of action and acceptable tolerability.

Up to now, visceral effects of BTX-A were clinically investigated mostly in painful bladder and pelvic syndromes (reviewed by Russell et al. (2013) and Jhang et al. (2014)). In addition, a few case reports pointed toward the beneficial effect of BTX-A in non-cardiac chest pain (Maradey-Romero and Fass, 2014) and perineal pain (Lim et al., 2010). Experimentally, the antinociceptive effect of BTX-A was investigated only in cystitis (Chuang et al., 2004; Coelho et al., 2014) and prostatitis (Chuang et al., 2008). In all listed reports and studies, the antinociceptive effect of primarily local BTX-A injections was examined.

Here we report that intrathecal, but not local and systemic, BTX-A application reduces pain as well as referred hyperalgesia and spinal c-Fos expression in experimental models of acetic acid induced peritonitis and capsaicin induced colitis.

Abbreviations: BTX-A, botulinum toxin type A; CNS, central nervous system; CSF, cerebrospinal fluid; GABA, γ-aminobutyric acid; i.c., intracisternal; i.col., intracolonic; i.p., intraperitoneal; i.t., intrathecal; NGS, normal goat serum; NSAIDs, Non-steroidal anti-inflammatory drugs; PBS, phosphate buffered saline; PBST, PBS+0.25% TritonX-100; RVM, rostral ventromedial medulla; SNAP-25, SyNaptosomal Associated Protein of 25 kiloDaltons

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2. Materials and methods

2.1. Animals

Experiments were carried out on male Wistar rats (300–400 g; University of Zagreb Medical School, Croatia). Animals were housed in a 12 h light/dark cycle with food and water available ad libitum, except during behavioral testing. Experiments were conducted according to the European Communities Council Directive (2010/63/EU). Care and handling of the animals were in accordance with the recommendations of the International Association for the Study of Pain (Zimmermann, 1983). Efforts were made to minimize the number of animals used. Study design, experimental protocols, and descriptions of animal treatment are closely followed by ARRIVE guidelines. Experiments were approved by the Ethical Committee of the University of Zagreb Medical School (Permit no. 07-76/2005-43).

2.2. Drugs

The following drugs and chemicals were used: BTX-A (Botox®, Allergan, Inc., Irvine, USA); acetic acid (Kemika, Zagreb, Croatia); capsaicin (Sigma, St. Louis, MO, USA); chloral hydrate (Merck KGaA, Darmstadt, Germany); ethanol (Kemika, Zagreb, Croatia); Tween 80 (Sigma, St. Louis, MO, USA) and petroleum jelly (Kemig, Zagreb, Croatia).

Each vial of Botox® contains 100 U (~4.8 ng) of purified *Clostridium botulinum* type A neurotoxin complex. To attain the needed doses, all drugs were diluted or dissolved in 0.9% saline, except capsaicin, which was dissolved in 0.9% saline containing 10% Tween and 10% ethanol.

Doses selected to test antinociceptive effect were chosen based on preliminary experiments and published data in models of somatic pain (Cui et al., 2004; Bach-Rojecky et al., 2010).

The following chemicals were used for the immunohistochemistry: paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA), Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA), normal goat serum (Vector, Inc., Burlingame, CA, USA), c-Fos rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc., CA, USA), goat anti-rabbit Alexa Fluor-448 (Invitrogen, Carlsbad, CA, USA), anti-fading agent (Fluorogel, Electron Microscopy Sciences, Hatfield, PA, USA).

2.3. Drug administration

2.3.1. Intrathecal (i.t.) injection

A small skin incision (2 cm) was performed at the lumbar L4/L5 level. BTX-A (2 U/kg) or 0.9% saline was injected between the vertebrae in a volume of 20 μl and the skin was sutured; correctness of application was verified by the animal's tail or hind limb brisk move.

2.3.2. Intracisternal (i.c.) injection

The animals were placed in a position in which the posterior neck area was easy to reach. The needle was carefully advanced between the occipital protuberance and the spine of the atlas to the cisterna magna, and BTX-A (2 U/kg) or 0.9% saline was injected in a volume of 20 μl; correctness of application was verified by extraction of a small amount of cerebrospinal fluid (CSF).

2.3.3. Intracolonic (i.col.) instillation

BTX-A (10 U/kg) or 0.9% saline in a volume of 1 mL were guided 7 cm proximally from the anocutaneous line, via the anus with a transparent 1 mm diameter cannula.

The animals were anesthetized with i.p. injection of chloral hydrate (300 mg/kg) during the i.t., i.c. and i.col. administration.

2.3.4. Intraperitoneal (i.p.) injection

BTX-A (15 U/kg) or 0.9% saline were injected in a volume of 10 mL/kg to conscious, gently restrained animals.

2.4. Behavioral testing

Before testing, the animals were habituated to the testing area (plastic cage/plastic cage with a wire mesh floor) for 30 min. Each experimental group contained 5–6 animals.

2.4.1. Acetic acid induced visceral pain model ("The writhing test")

The animals were i.p. injected with 10 ml/kg of 1% acetic acid and immediately returned to the transparent cage for a 1 h observation period, as described by Koster et al. (1959). Pain was measured as the number of abdominal writhes. A writhe is defined as arching of the back, extension of hind limbs and contraction of abdominal musculature. Measurements were conducted 5 days after i.c. and i.p. BTX-A or 0.9% saline injection and 2 days after the i.t. injections.

2.4.2. Capsaicin induced visceral pain model

The model was originally developed for mice (Laird et al., 2001), and later adapted for use in rats (Sanoja et al., 2010). In brief, 200 μl of 0.1% capsaicin was administered into the colon, 7 cm proximally from the anocutaneous line, via the anus, with a transparent 1 mm diameter cannula. Before i.col. capsaicin instillation, petroleum jelly (Vaseline) was applied on the perianal area to avoid direct contact with the irritant. Control animals were subjected to the same treatment, but instead of capsaicin, received 0.9% saline. Animals injected with i.t. BTX-A or 0.9% saline were tested after 2 days, while those i.col. instilled, after 5 days.

Immediately after i.col. instillation of 0.1% capsaicin or 0.9% saline, the animals were returned to the transparent cage for a 20 min observation period where spontaneous behavior was observed and counted. Visceral pain behavior was considered licking and contraction of the abdomen, stretching, hump-backed position and hunching (Sanoja et al., 2010). After 20 min, referred mechanical hyperalgesia was tested by using a series of von Frey filaments (Stoelting Co, Wood Dale, IL, USA) ranging from 1 g to 26 g, in ascending order, to the abdominal area with the exception of genital area. Repeated stimulation of the same area was avoided to prevent sensitization. The lowest filament that elicited a withdrawal response was considered the threshold stimulus. Each filament was applied 3 times, kept in bent position for 2 s and with an inter-stimulus interval of 5–10 s.

2.5. Immunohystochemical analysis

An immunohystochemical analysis was performed as previously described (Drinovac et al., 2013) on samples collected from the capsaicin induced visceral pain model. The number of c-Fos was counted in the superficial sensory laminae of the spinal cord dorsal horn (I and II) at the sacral S3/S2 section.

2.6. Statistical analysis

Results, presented as mean ± S.D., were analyzed by one-way analysis of variance followed by Tukey's post-hoc test. $P < 0.05$ was considered significant.