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Oxytocin inhibits the rat medullary dorsal horn Sp5c/C1 nociceptive transmission through OT but not V_{1A} receptors

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ABSTRACT

The medullary dorsal horn (MDH or Sp5c/C1 region) plays a key role modulating the nociceptive input arriving from craniofacial structures. Some reports suggest that oxytocin could play a role modulating the nociceptive input at the MDH level, but no study has properly tested this hypothesis. Using an electrophysiological and pharmacological approach, the present study aimed to determine the effect of oxytocin on the nociceptive signaling in the MDH and the receptor involved. In sevoflurane, anesthetized rats, we performed electrophysiological unitary recordings of second order neurons at the MDH region responding to peripheral nociceptive-evoked responses of the first branch (V1; ophthalmic) of the trigeminal nerve. Under this condition, we constructed dose-response curves analyzing the effect of local spinal oxytocin (0.2–20 nmol) on MDH nociceptive neuronal firing. Furthermore, we tested the role of oxytocin receptors (OTR) or vasopressin V_{1A} receptors (V_{1A}R) involved in the oxytocin effects. Oxytocin dose-dependently inhibits the peripheral-evoked activity in nociceptive MDH neurotransmission. This inhibition is associated with a blockade of neuronal activity of Aδ- and C-fibers. Since this antinociception was abolished by pretreatment (in the MDH) with the potent and selective OTR antagonist (L-368,899; 20 nmol) and remained unaffected after the V_{1A}R antagonist (SR49059; 20 nmol or 200 nmol), the role of OTR is implied. This electrophysiological study demonstrates that oxytocin inhibits the peripheral-evoked neuronal activity at MDH, through OTR activation. Thus, OTR may represent a new potential drug target to treat craniofacial nociceptive dysfunction in the MDH.

1. Introduction

The medullary dorsal horn (MDH) is involved in pain processing from the craniofacial area (Hu et al., 2005; Mørch et al., 2007; Tsai et al., 1999) and modulates nociceptive information arriving from the first branch of the trigeminal nerve (V1, ophthalmic branch) (Meng et al., 1997; Malick et al., 2000). This branch nerve innervates the periorbital area, intranasal mucosa and supratentorial dural and pial tissues (Shankland, 2001). Noxious stimuli carried by this branch through Aδ- and C-fibers arrive at the MDH where they contact second order neurons projecting to supraspinal sites (Hu, 1990; Shimizu and Suzuki, 2011). At this level, the nociceptive information can be endogenously or exogenously modulated by an array of neuromediators (Akerman et al., 2011; Goadsby et al., 2009). Indeed, some drugs are therapeutically relevant; for example, the 5-$HT_{1B/1D}$ receptor agonists (triptans) and CGRP receptor antagonists (gepants), both of which are specific anti-migraine compounds that selectively inhibit the nociceptive input arriving at MDH or Sp5c/C1 region (Kaube et al., 1993; Storer et al., 2004).

A research in humans with tension-type headache or migraine has suggested that intranasal oxytocin seems to have analgesic effects (Wang et al., 2013); regardless, the *locus* of action and receptors involved remain unknown. A potential role of oxytocin at MDH level is suggested considering that after intranasal administration of ^{125}I-labeled oxytocin in rats, several areas involved in pain transmission, including Sp5c are marked (Tzabazis et al., 2017). Although this neuropeptide has emerged as an interesting molecule with analgesic properties when given spinally (at lumbar level in humans) (Condés-Lara et al., 2016), the receptors involved

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remain unclear (González-Hernández et al., 2014). Hence, the potential antinociceptive actions of oxytocin and receptors implicated in MDH neurotransmission deserves attention.

Considering that oxytocin binds to both OT receptors (OTR; pK_i: 8.2) and vasopressin V_{1A} receptors (V_{1A}R: pK_i: 8.3) with similar affinities (Alexander et al., 2015), the present study was designed using an electrophysiological approach to elucidate the neuropharmacological basis of the following: (i) the effect of oxytocin on Sp5c/C1 nociceptive responses evoked by electrical stimulation of the supraorbital dermatome (which is mostly innervated by the V1 branch); and (ii) the role of OTR or V_{1A}R in the oxytocin-induced inhibition by analyzing the effects of the selective and potent antagonists L-368,899 (OTR; pK_i: 8.1, Williams et al., 1994) or SR-49059 (V_{1A}R; pK_i: 8.1–9.3, Serradeil-Le Gal et al., 1993). Our results show that at MDH, oxytocin inhibits the peripheral nociceptive input through OTR but not through V_{1A}R.

2. Material and methods

2.1. Animals

A total of 56 adult male Wistar rats (260–320 g) provided by our institutional animalarium were used. The animals were housed for at least four days in plastic cages (1 rat per cage) with on wood-based bedding, controlled temperature (23 ± 2 °C) and lighting (12/12-h light-dark cycle; light beginning at 7 a.m.), with access to food and water *ad libitum*. Our Institutional Ethics Committee approved all animal procedures and we followed the regulations established by the Official Mexican Standard (NOM-062-ZOO-1999), which fully complies with (i) the ARRIVE guidelines and (ii) the guide for the Care and Use of Laboratory Animals established by the National Institutes of Health (NIH Publications No. 8023, revised 1978).

2.2. General methods

2.2.1. Surgical procedures

Animals were introduced into a chamber and deeply anesthetized with 6% (v/v) sevoflurane delivered through a vaporizer at 3:1 ratio of N_2O and O_2. The adequacy of anesthesia before surgery was judged by the absence of ocular reflexes, a negative tail flick test and corporal relaxation. Under these conditions, an intratracheal cannula was inserted for artificial ventilation (56 strokes/min) and to maintain the anesthesia throughout the experiments. The stroke volume was adjusted to maintain a normal acid-base equilibrium. Rats were mounted on a stereotaxic frame (Kopf Instruments, Tujunga, CA). The muscles of the dorsal neck were separated to access the MDH. A cervical (C1) laminectomy was performed and the dura was carefully removed to expose the brainstem, at the level of the caudal medulla oblongata. The subsequent protocols were performed under 2.0% sevoflurane to achieve the ethically adequate level of anesthesia without depressing neuronal responses to noxious stimuli. The animals where paralyzed with a single pancuronium bromide injection (2 mg/kg; intramuscular). End-tidal CO_2 was monitored (Capstar-100, CWI Inc., Ardmore, PA, USA), and core body temperature was maintained at 38 °C using a circulating water pad. At the end of each experiment, animals were perfused transcardially (see Section 2.3).

2.2.2. Extracellular unitary recordings at medullary dorsal horn

Extracellular unitary recordings of MDH neurons with input from the supraorbital receptive field were made using quartz-insulated platinum/tungsten microelectrodes (4–9 MΩ) mounted in a microdrive Eckhorn system (Thomas Recording GmbH, Giessen, Germany). This device allows the insertion of 3 microelectrodes (distance inter-electrode: 300 μm) into neural tissue and each microelectrode was advanced into the MDH independently using the Eckhorn Matrix multiuser software (Thomas Recording GmbH, Giessen, Germany). In most cases we recorded only one extracellular unit per animal and only in four animals we recorded two extracellular signals (see 3.1 section). To search for single-unit discharges, the microelectrodes were lowered (400–1600 μm from the surface) in small steps (2–5 μm). A first search of cells responding to tactile stimulation of the ophthalmic dermatome was performed. In all cases, the specific receptive field was exclusively assessed in the first division of the trigeminal nerve (V1; ophthalmic). The receptive field was assessed for non-noxious (brushing) and noxious (pinching) input; then, electrical stimulation was applied by two electrodes inserted into the receptive field. In this case, two fine needles (27 G) attached to a stimulus isolator unit were inserted subcutaneously into the receptive field of the recorded neuron. Then, electrical test stimulation was conducted. This test consists of 20 stimuli at 0.5 Hz with 1-msec pulse duration at 1.5 times the threshold intensity (0.1–3 mA) required to evoke a Aδ- and C-fiber response.

The evoked extracellular neuronal activity was recorded, amplified, digitalized, and discriminated using CED hardware and Spike2 v5.15 software (Cambridge Electronic Design). Raw and discriminated signals were fed through an audio monitor and displayed on an oscilloscope. Waveforms were stored on a hard drive for off-line analysis. Baselines and evoked activities of the MDH wide dynamic range (WDR) neurons were recorded and analyzed as post-stimulus time histograms (PSTH) to detect the occurrence of statistically significant neuronal responses. On this basis, the stimulating threshold to evoke action potentials and their frequency of occurrence, resulting from the stimulation of the peripheral receptive field located on the supraorbital area, were attributed to the recruitment of Aδ- and C-fibers. Considering the distance between the receptive field and the recording electrode, the peak latencies observed correspond to peripheral conduction velocities within the Aδ- (3–25 msec) and C-fibers (25–80 msec) (see Fig. 1 for details). Thus, the number of action potentials that occurred in response to 20 receptive field stimuli was compared before (basal) and after vehicle/drug treatment.

The evoked neuronal responses were evaluated after oxytocin (0.2, 2 and 20 nmol; n = 6 cells, each concentration) or vehicle (n = 6 cells) administration at 10, 20, 30, 40, 50 and 60 min. The role of OTR or V_{1A}R in the oxytocin effect was also evaluated using the competitive antagonists L-368,899 (20 nmol; n = 6 cells) or SR-49059 (20 or 200 nmol; n = 6 cells each group) (Pettibone et al., 1993; Serradeil-Le Gal et al., 1993). The antagonist was administered 5 min before oxytocin or vehicle. All drugs/vehicles were carefully delivered at spinal level (topical) where the microelectrodes were positioned in a volume of 20 μl using a Hamilton® syringe.

Finally, it was of the foremost importance to ascertain if MDH administration of OTR (n = 6 cells), V_{1A}R (n = 6 cells) antagonist or its vehicle (n = 6 cells) was devoid of effect *per se* on the nociceptive-evoked responses; accordingly, any effect of a given antagonist on the oxytocin-induced inhibition is due to a direct interaction of the antagonist with its respective receptor.

2.3. Histological reconstruction of recording sites

At the end of the electrophysiological experiments, the animals received an overdose of sevoflurane (8% v/v) and were transcardially perfused with saline solution followed by 10% formaldehyde (~200 ml of each). Brains were post-fixed in 10% formaldehyde and cut into 40-μm serial coronal sections with a freezing microtome (Leica Instruments BmbH, Nussloch, Germany). The position of the cells was observed with a light microscope.