PAIN® 154 (2013) 1449–1456

www.elsevier.com/locate/pain

Neurohormonal effects of oxytocin and vasopressin receptor agonists on spinal pain processing in male rats

Pierre-Eric Juif, Pierrick Poisbeau *

Centre National de la Recherche Scientifique and University of Strasbourg, Institut des Neurosciences Cellulaires et Intégratives, Research Group "Molecular Determinants of Pain", Strasbourg, France

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

ARTICLE INFO

Article history:
Received 26 April 2013
Accepted 1 May 2013

Keywords:
Arginine vasopressin
Central sensitization
In vivo single unit recording
Nociception
Oxytocin
Spinal cord
Stress-induced analgesia

ABSTRACT

Oxytocin (OT) and arginine vasopressin (AVP) are 2 neuropeptides that display well-known effects on the reproductive system. Although still controversial, oxytocin and vasopressin were demonstrated to exert potent effects on the nociceptive system when administered directly in various central nervous structures. On the other hand, little is known about their peripheral (hormonal) actions on nociception and pain responses. The aim of the present work was to characterize the effects of physiological blood concentrations of OT and AVP on spinal nociception and on pain responses. To do so, growing doses of OT or AVP were administered intravenously and the nociceptive processing by spinal cord neurons was analyzed in anesthetized male rats in vivo. We observed that the action potentials mediated by C-type nociceptive fibers was strongly reduced (antinociception) after intravenous injections of low doses of OT (<5 μg) or AVP (<500 pg), whereas an increase (pronociception) was observed at higher doses. Interestingly, antinociceptive and pronociceptive effects were fully abolished in the presence of the OT receptor antagonist and the AVP receptor antagonist type 1A (V_{1A}), respectively. We confirmed this result with a behavioral model of forced swim stress-induced analgesia associated with plasmatic release of OT (and not vasopressin). Stress-induced analgesia was transiently lost after i.v. administration of OTR antagonist. Together, the present work provides straightforward evidence that blood levels of OT and AVP modulate nociception, windup plasticity and pain responses. The final target structures explaining these effects remains to be identified but are likely to be C-type nociceptors.

1. Introduction

Oxytocin (OT) and arginine vasopressin (AVP) are 2 nonapeptides synthesized in the paraventricular (PVN), the supraoptic and the accessory magnocellular nuclei of the hypothalamus [41–43]. Axons of the magnocellular neurons project to the posterior hypophysis and secrete their content in the blood flow (hormonal action). On the other hand, OT contained in parvocellular neurons of the PVN plays a neuromodulatory role after its secretion in various central nervous system (CNS) structures. OT and AVP exert their neuroendocrine and neuromodulatory roles after binding to G protein–coupled receptors: the oxytocin receptor (OTR) and the vasopressin receptors (V_{1A}R, V_{1B}R, V_2R), which are expressed by peripheral tissues (eg, uterus/testis, breast, heart) and many CNS structures [15]. OT and AVP, as well as their receptors, exhibit a high degree of sequence homology and both neuropeptides can activate OTR (Kd: OT = 1–2.5 nM; AVP = 1.7 nM) and V_{1A}R (Kd: OT = 78 nM; AVP = 0.28 nM) [6,17,38].

OT has been described to display analgesic effects after administration in the CNS [9,13,14,29,52,59]. Activation of oxytocinergic axons produces a potent OTR-mediated antinociception because a remarkable colocalization between these neurons and the OTR binding sites has been described in superficial laminae of the spinal cord dorsal horn [23,33,47,48,51]. OT action seems to amplify GABAergic inhibition leading to a selective decrease in excitability in superficial [4] and deep dorsal horn neurons [7,8,36] integrating nociceptive messages. It has been recently suggested that the hormonal role of OT or AVP on nociceptive processing occurs in dorsal root ganglion (DRG) because OTR are expressed by the unmyelinated nociceptive C fibers at this level [31]. Contrary to OT action, AVP modulations of pain are still unclear but mostly rely on the signaling by neuronal V_{1A}R [39]. AVP-containing PVN neurons could target various supraspinal structures [55,57,58] and modulate nociceptive responses. In humans, AVP release is placed under a tight regulation by opioids [37], suggesting an analgesic role. However, in rats, intrathecally injected AVP fails to produce hyponociception in the tail flick test [28].

* Corresponding author. Address: INCI—UPR 3212 CNRS, 21 rue René Descartes, 67084 Strasbourg, France. Tel.: +33 3 68 85 14 76; fax: +33 3 88 61 33 47.
E-mail address: poisbeau@inci-cnrs.unistra.fr (P. Poisbeau).

0304-3959/$36.00
http://dx.doi.org/10.1016/j.pain.2013.05.003

Central and peripheral secretions of OT and AVP occur under several pathophysiological conditions, eg, after neuropathy [27] or forced swim stress [30]. Because OT exerts an analgesic role at peripheral level after systemic administration [25,32], it has been hypothesized that elevation of plasmatic OT exerts a role in forced swim stress–induced analgesia [22,35]. The peripheral role of OT and the underlying mechanisms on nociceptive processing still remain unidentified. Similar to OT, systemic injections of AVP also lead to analgesia [30]. A recent study further indicated that AVP- and OT-induced antinociception was mediated by the recruitment of $V_{1A}R$ in mice. Indeed, intraperitoneal injections of OT or AVP produce analgesia in $OTR^{-/-}$ mice but do not alter nociceptive threshold in $V_{1A}R^{-/-}$ mice [40].

In the present work, we investigated the hormonal effects of OT and AVP and the role of their receptors on spinal nociceptive processing and central sensitization in rats.

2. Materials and methods

Male Sprague Dawley rats (250–350 g; Janvier, Le Genest St Isle, France) were used for this study. They were housed by group of 4 under standard conditions (room temperature [22°C], 12–12 h light–dark cycle) with ad libitum access to food and water. All experiments were conducted in conformity with the recommendations of the European Union directive on animal experimentation (2010/63/EU adopted on September 22, 2010) and were evaluated by the regional ethic committee in charge of animal experimentation (CREMEAS, authorization AL 01/01/02/11). This study was conducted under the responsibility of authorized personnel (license 67-116 from the French Department of Agriculture to PP).

2.1. Surgical procedure for catheter implantation

Implantation of catheters in the jugular vein was performed under isoflurane anesthesia (3% in pure oxygen at a flow rate of 1 L/min). The jugular vein was exposed, and a small incision was made in the vessel with iridectomy scissors. The catheter (PE50, Warner Instruments, Hamden, USA) filled with saline and soaked at the tip in heparin (Heparin-Natrium Braun, 10,000 IU/mL) was inserted into the vein and ligated to the vessel. In the behavioral part of this work, the catheter was subcutaneously tunneled so that only a small part of it emerged from the animal's neck. The wounds were loosely closed with a suture (Silkam 4-0, Unodis, Haguenau, France). Rats were allowed to recover for 5 days after surgery before being submitted to swim stress.

2.2. In vivo electrophysiology

Single unit extracellular recordings were made from dorsal horn neurons in the lumbar enlargement of the spinal cord of the rat (Fig. 1) following the procedure previously described by Urch and Dickenson [49]. Rats were anaesthetized with isoflurane (3% in pure oxygen; flow rate, 1 L/min; Vaporizer Isotec 3 datex-Ohmeda) during spinal surgery, and concentration could be lowered to 1.5% during electrophysiological recordings. This was sufficient to ensure proper maintenance of the anesthesia and areflexia, a prerequisite to obtain stable and long-lasting electrophysiological recordings. After implantation of the catheter, a laminectomy was performed to expose the L4–L5 segments of the spinal cord. The cord was then firmly attached by vertebral clamps on a stereotaxic frame, caudal and rostral to the exposed section. Before recording, the meninges were delicately removed, and the spinal cord surface was covered with a thin layer of mineral oil. Single-unit extracellular recordings were made with a stainless steel electrode (FK#02; FHC, UK) connected to a differential amplifier (DAM80, WPI). An electrode was lowered into the dorsal horn to record neurons located in the deep dorsal horn of the spinal cord. Data were acquired and analyzed by a CED 1401 analog-to-digital interface coupled to a computer with Spike 2 software (Cambridge Electronic Design, Cambridge, UK). All neurons included in the present study responded to both innocuous and noxious stimuli after electrical stimulation of the peripheral hind paw receptive field and were located in the medial part of the deep layers of the dorsal horn (809 ± 32.1 μm; $n = 32$; Fig. 1A). They corresponded to wide dynamic range neurons and exhibited windup plasticity (ie, increase of action potential discharge frequency) after intense repetitive stimulation of the peripheral receptive field. After stimulation of the receptive field, the recorded neuron emitted action potentials (APs) corresponding to the activation of fast-conducting Aβ (delay to stimulus artifact <20 ms), slow-conducting Aδ (delay of 20–90 ms) and very slow-conducting C fibers (delay of 90–300 ms). APs observed 300 to 800 ms after the stimulus artifact were considered as being part of the postdischarge. We first adjusted the stimulation to 1.5 times the C-type fiber threshold and the frequency to 0.2 Hz (pulse duration 1 ms) to avoid windup phenomenon in the recorded neurons. To trigger central sensitization and analyze the modulation of windup discharges, electrical stimulation was then set at 3 times the threshold for C fibers (pulse duration 1 ms) and applied every second (1 Hz). The AP discharge after each stimulation of the receptive field could be monitored in raster plots (Fig. 1C, middle graph) and poststimulus histograms were built (Fig. 1C, lower histogram) by counting the number of APs per time periods of 10 min (ie, 120 stimulations at a frequency of 0.2 Hz; stimulus intensity of 1.5 times C-fiber threshold; pulse duration of 1 ms). AP changes were compared before and immediately after the injection of OT and AVP receptor agonists and

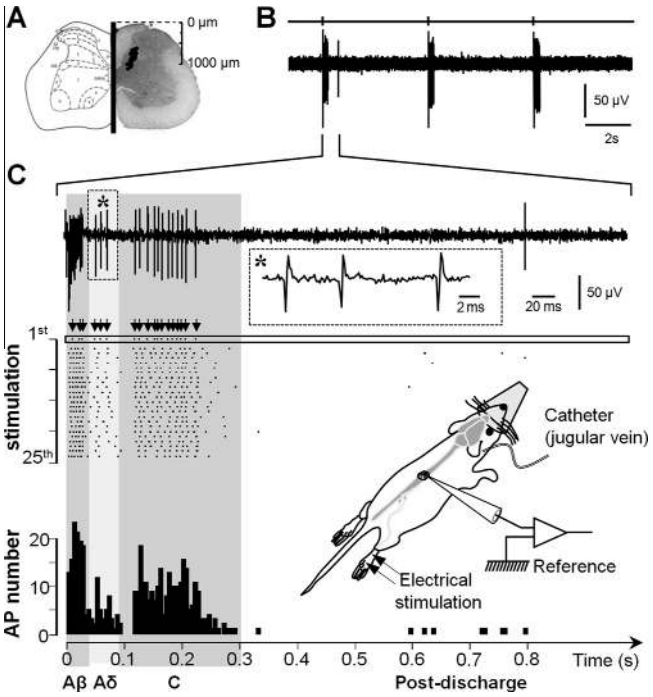

Fig. 1. (A) Localization of the recorded cells using the experimental setup drawn in (C). Each black dot represents a recorded neuron. (B) Representative trace illustrating single unit extracellular voltage response of a wide dynamic range neuron after 3 electrical stimulation of the receptive field (1.5 times the threshold for C fibers). (C) Upper trace is a magnified view of the AP discharge after the first electrical stimulation. Each AP was detected and shown in a raster plot (middle graph: 1 AP = 1 dot; here for 25 successive stimulations). The bottom histogram shows the number of APs per time bins after the stimulus artifact. This allows to count the total number of APs on the basis of their conduction velocity: Aβ (0–20 ms), Aδ (20–90 ms), C-fiber (90–300 ms) and C-fiber-evoked postdischarge (300–800 ms) responses.

antagonists. To quantify windup, we counted the total number of APs per fiber type during time periods corresponding to 30 stimulations before and after the treatments. The number of APs was also counted after every stimulation and helped estimate the time required to reach the plateau phase (windup efficacy measured through the slope) in each pharmacological condition.

2.3. Forced swim stress and measurement of mechanical nociceptive thresholds

The forced swimming apparatus consisted in a Plexiglas cylinder (diameter 30 cm, wall height 60 cm), filled with water (height 40 cm) at a temperature of 20°C. Five days after a surgery aimed at installing chronic intravenous catheters, rats were individually forced to swim in the apparatus for 10 min. This protocol of stress-induced analgesia was chosen because it is associated with a plasmatic release of OT but not of AVP [54].

Mechanical nociceptive thresholds have been measured with von Frey filaments (OptiHair, Marstock NervTest, Germany). To do so, habituated rats were placed in Plexiglas cages (23 × 17 × 14 cm; Ugo Basile, Italy) with wire mesh bottoms. Each monofilament was placed perpendicularly onto the midplantar region of the hind paw and pressure was increased until the point of deflection of the filament was reached. Nociceptive pressure threshold corresponded to the filament force, which induced an aversive behavior, evident as a fast withdrawal, licking or shaking. This measurement followed the ascending and descending procedure [5] with forces ranging from 8 to 256 mN (11 logarithmic steps). This test was repeated 3 times for each paw and values were averaged. The mechanical nociceptive threshold was measured at 3 different time points for each animal—before the swim session (control threshold), 10 min after the swim session (stress-induced analgesia) and 5 min after the intravenous injection (~25 min after swim)—to reveal any effect of the drug on stress-induced analgesia.

2.4. Drugs and treatments

We used [Thr^4,Gly^7]-oxytocin (TGOT; Sigma-Aldrich, France) and [Arg^8]-vasopressin (Bachem, Weil am Rhein, Germany), as selective agonists for OT and AVP receptors, respectively. Agonists were prepared as 1000 times concentrated stock solutions in a molar chlorohydrate (HCl) solution 0.25% (v/v) in saline (NaCl 0.9%). From this stock solution, drugs were diluted and injected i.v. in a range between 10^{-10} M and 10^{-5} M. Final concentration of HCl in saline was of 800 nM to 8 pM, for the maximal and minimal concentration of agonist injected. Vehicle containing the maximal concentration of HCl was used in control conditions (referred to as saline) and had no effects in electrophysiological or behavioral experiments. The selective antagonists for OTR and V_{1A}R were, respectively, d(CH$_2$)$_5$-[Tyr(Me)^2,Thr^4,Orn^8,des-Gly-NH$_2$^{9}]-vasotocin (dOVT; Bachem, Germany) and [Phenylacetyl^1,O-Me-D-Tyr^2,Arg^{6,8},Lys^9]-vasopressin amide (AV_{1A}R: Sigma Aldrich, Saint-Louis, France). They were prepared in the same vehicle than the agonist. Intravenous administration of the OT and AVP receptor ligands consisted in a bolus of 500 μL of the drug infused during 30 s.

2.5. Statistical analysis

Data are expressed as mean ± SEM. One-way ANOVA with repeated measures followed by a Bonferroni post hoc test (Statistica, Tulsa, OK, USA) was used to analyze the effects of the treatments on spinal nociceptive processing and on windup properties. Friedman test followed by a Dunn's multiple comparison test was carried out in order to assess the effects of the pharmacological treatments on swim stress induced analgesia (von Frey, in mN). Statistical significance was set at $P < .05$.

3. Results

3.1. Changes in nociceptor activation threshold after i.v. injection of TGOT and AVP

In the first set of experiments, we characterized the systemic effects of growing doses of TGOT (from 5 ng to 50 μg) and AVP (from 50 pg to 50 ng) on the excitability of spinal cord neurons located in the deep dorsal horn.

We first measured the stimulation threshold required to observe APs resulting from the activation of Aβ, Aδ and C-type sensory neurons. Thresholds to observe APs associated with the activation of Aβ and Aδ were, respectively, 11 ± 2 V ($n = 7$) and 17 ± 4 V ($n = 5$). These thresholds for A-type fibers remained similar at all doses tested. This was not the case for C-fiber thresholds, which were modulated with various doses of TGOT. Compared to control threshold (saline: 29 ± 3 V; $n = 7$), TGOT increased the C-fiber threshold after i.v. injection ($n = 7$ neurons per dose) at 50 ng (45 ± 5 V; $P < .05$), 0.5 μg (41 ± 11 V; $P < .05$) and 5 μg (41 ± 11 V; $P < .05$). At the highest dose tested, a slight but not significant decrease was observed (50 μg: 27 ± 3 V; $P > .05$). As for TGOT, A-type activation thresholds remained unaffected after injection of AVP at all doses. Compared to control values (saline: 27 ± 2 V, $n = 7$ recorded neurons per condition), C-fiber threshold was only slightly decreased (but not significantly) at AVP doses of 50 pg (26 ± 2 V), 500 pg (23 ± 3 V), 5 ng (24 ± 3 V), 50 ng (22 ± 4 V) and 500 ng (23 ± 9 V).

3.2. Dose-dependent modulation of spinal nociceptive processing by TGOT and AVP

TGOT and AVP effects on spinal nociceptive processing were characterized by quantifying changes in the number of APs per fiber type (Aβ, Aδ, C + postdischarge) for time periods of 10 min, ie, after 120 stimulations of the receptive field at an intensity of 1.5 times the C-type fiber threshold (frequency: 0.2 Hz; pulse duration: 1 ms). Compared to a control period (no injection), injecting the vehicle (saline) had no effect on the total mean number of AP per fiber type. No changes were observed while analyzing the frequency of AP corresponding to A-type fibers after injection of TGOT or AVP, at all doses tested. In contrast, only changes in the number of AP corresponding to the recruitment of C-type sensory fibers, contributing also to the postdischarge, were observed and at different ranges of concentration for TGOT and AVP.

As shown in Fig. 2A,B, clear and significant antinociceptive effects of TGOT (reduction in the number of APs) could be observed after i.v. injection of TGOT at 50 ng (Fig. 2B, −46.6 ± 6.7%, $n = 7$; $P < .001$) and 500 ng (−39.9 ± 6.0%, $n = 5$; $P < .001$). Interestingly, an increase of the firing was also induced after i.v. injections of TGOT at a higher dose of 5 μg (48.9 ± 17.2%, $n = 6$; $P < .05$). This dual anti- and pronociceptive profile was also observed for AVP, but at a different concentration range (Fig. 2C,D). At a low dose (50 pg), AVP slightly reduced (but not significantly) the number of AP corresponding to the activation of C fibers (−20.0 ± 8.7%, $n = 6$; $P = .058$) whereas at higher doses, a clear increase was observed after AVP injection at 5 ng (63.8 ± 11.0%, $n = 6$; $P < .001$) and 50 ng (Fig. 2D, 57.5 ± 16.3%, $n = 6$; $P < .01$). Together, it is interesting to note that at a concentration of 50 ng, TGOT and AVP may have opposite effect on spinal nociceptive processing.

3.3. Receptors involved in TGOT and AVP-induced nociceptive modulation

We next investigated the contribution of OTR and V_{1A}R in the modulation of nociceptive processing by coinjecting i.v. the

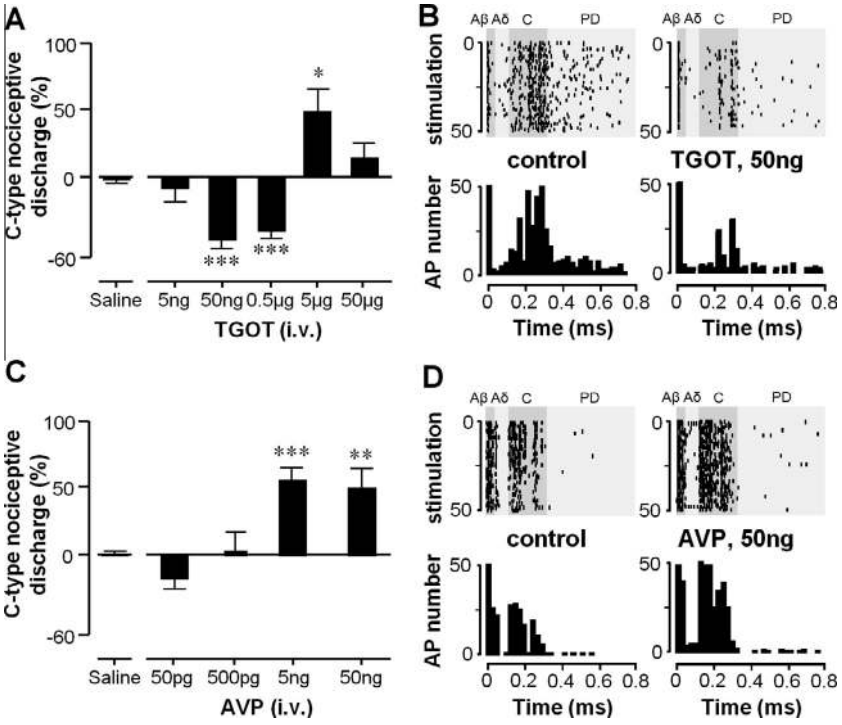

Fig. 2. (A,C) Histograms representing the dose response effects of TGOT (A) and AVP (C) on C-fiber-related AP discharge. Dose injected i.v. are indicated and the percentage of change compared to control (no injection) is shown. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ (ANOVA followed by a Bonferroni test; comparison of saline vs treatment). (B,D) Raster plot and histogram illustrating the effects of TGOT (B) and AVP (D) at a concentration of 50 ng. Note that only the C-fiber-related AP discharge is affected by TGOT or AVP.

different antagonist with TGOT or AVP (Fig. 3). Injections of TGOT at 50 µg or of AVP at 50 pg were previously associated with an antinociceptive effect (Fig. 3A), ie, a reduction in the number of C-fiber-related AP number after receptive field stimulation (TGOT −46.6 ± 6.7%, $n = 7$; AVP −20.0 ± 8.7%, $n = 6$). If the OTR antagonist dOVT (50 µg) was coinjected with either TGOT or AVP, the reduction in the number of C-fiber-related APs was fully abolished (TGOT + dOVT: −2.5 ± 5.6%, $n = 7$; $P < .001$; AVP + dOVT: 1.8 ± 2.1%, $n = 7$; $P < .05$). This indicated that the antinociceptive effects of OT and AVP at these concentrations were mediated by the activation of OTR. In contrast, coinjection with $V_{1A}R$ antagonist (50 µg) was without significant effect on antinociception induced by TGOT (−35.8 ± 3.2%, $n = 6$; $P > .05$) or AVP (−19.1 ± 4.1%, $n = 7$; $P > .05$). This result further confirmed that antinociception mediated by low concentration of TGOT and AVP was mediated solely by the recruitment of OTR.

A similar analysis was performed with pronociceptive concentrations of TGOT (5 µg) and AVP (50 ng), coinjected with antagonists of OTR or V_{1A}Rs, both injected at 50 µg (Fig. 3B). Under these circumstances and compared to TGOT (48.9 ± 17.2%, $n = 6$) and AVP injections alone (57.5 ± 16.3%, $n = 6$), we failed to see any modulatory effect of the OTR antagonist dOVT if coapplied with TGOT (29.3 ± 9.1%, $n = 6$; $P > .05$) or AVP (39.4 ± 15.7%, $n = 6$; $P > .05$). In sharp contrast, the $V_{1A}R$ antagonist (50 µg) fully blocked and even promoted an antinociceptive action if coinjected with TGOT (−32.7 ± 4.6%, $n = 6$; $P < .01$) or AVP (−9.1 ± 13.2%, $n = 6$; $P < .01$). This result likely suggested that pronociceptive effects of TGOT and AVP, at high doses, were mostly mediated by the activation of V_{1A}Rs.

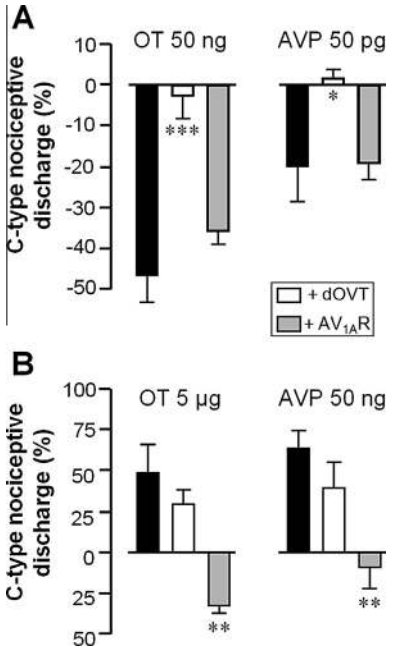

Fig. 3. Histograms illustrating the (A) antinociceptive and (B) pronociceptive effects of TGOT and AVP at different doses and the contribution of OTR and $V_{1A}R$ to these effects after blockade of these receptors by dOVT (white bars) and $AV_{1A}R$ (gray bars), respectively. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ (ANOVA followed by a Bonferroni test, comparisons agonist vs agonist + antagonist).

3.4. Contribution of OTR to stress-induced analgesia

In a previous study, release of OT and AVP has been studied in the hypothalamus and in the blood flow after a forced swim stress paradigm [54]. Interestingly, it was demonstrated that if OT and AVP concentrations were both increased after swim stress, only elevated OT levels could be detected in the blood. Because analgesia is observed after forced swim stress, we investigated whether this effect might be due to this increase in blood OT. While performing a measure of mechanical nociception with the von Frey

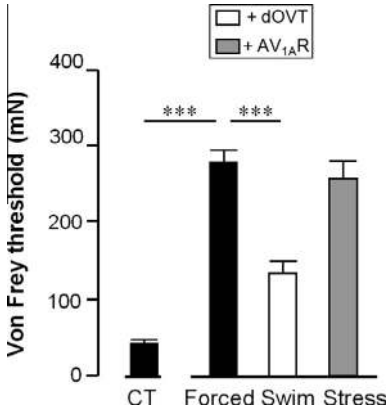

Fig. 4. Consequences of OTR and $AV_{1A}R$ antagonists on mechanical analgesia induced by a forced swim stress (FSS, 10 min, 20°C). Mechanical nociceptive thresholds were measured with the von Frey filaments in control (CT, before swim), 10 min after swim stress (black bar) and 25 min after swim stress: 5 min after intravenous infusion of the OTR (dOVT) and $V_{1A}R$ antagonists. ***$P < .001$. Statistical analysis was carried out by the Friedman test followed by Dunn's multiple comparison test.

test 10 min after the forced swim stress procedure (10 min at 20°C), we found a significant analgesia, as expected (Fig. 4). This was associated with an important increase in the mean mechanical nociceptive threshold from 128.7 ± 4.0 mN to 308.7 ± 11.9 mN ($n = 21$; $P < .001$).

Using the same animals, we then injected the OTR antagonist dOVT or the $V_{1A}R$ antagonist to determine the possible contribution of OTR or $V_{1A}R$ to this stress-induced analgesia. Administration of the $V_{1A}R$ antagonist did not change significantly the mechanical nociceptive threshold (291.79 ± 17.34 mN, $n = 9$; $P > .05$). Analgesia resulting from swim stress was, however, significantly reduced 5 min after i.v. injection of dOVT as indicated by the limited increase of the mean nociceptive threshold (199.50 ± 11.51 mN, $n = 9$; $P < .001$). This effect was selective because similar injection of the vehicle did not change the maximal analgesia ($n = 3$, data not shown). Occlusion of swim stress analgesia by dOVT was reversible and fully recovered after 24 h. Together, this experiment confirmed that OT receptor activation by circulating levels of endogenous OT not only produces spinal antinociception but contributes to analgesia after swim stress.

3.5. Consequences of peripheral OTR/$V_{1A}R$-mediated modulation on central sensitization

Central sensitization was classically initiated in the recorded wide dynamic range neurons by using an intense repetitive stimulation of the receptive field (frequency of 1 Hz, intensity 3 times the C-fiber threshold). As illustrated in Fig. 5 (left graphs), a train of repetitive stimulations triggered a progressive increase in the number of APs mediated by C fibers which reached a plateau phase after about 16 or 17 stimulations. No changes were observed for A fibers (Aβ or Aδ), as expected.

Using this experimental model, we confirmed that a low dose of TGOT (50 ng) produced significant antinociceptive effects (ie, C-type fiber mediated) through OTR activation when injected in the blood flow (Fig. 5A). No changes in the number of APs carried by A-type fibers were observed while testing the different concentration of TGOT and the OT/V_{1A} receptor antagonists. Compared to the control condition (saline), we observed a reduction in the time required to reach the windup plateau phase (ie, slope reduction shown in Fig. 5A, middle graph; control: 3.86 ± 0.27; TGOT 2.83 ± 0.24; $n = 5$; $P < .01$) and a reduction in the total number of AP during windup (Fig. 5A, right graph: −55.2 ± 1.9%, $n = 5$, $P < .001$). Windup reduction was still observed when TGOT was coinjected with AV_{1A} (−30.3 ± 6.9%, $n = 5$, $P > .05$ compared to TGOT alone) but was fully blocked in the presence of dOVT (13.0 ± 5.0%, $n = 5$, $P < .05$ compared to TGOT alone). Similar conclusions were drawn while analyzing windup slope changes (Fig. 5A, left and middle graphs).

On the other hand, blood injection of TGOT at a pronociceptive dose of 5 μg (Fig. 5B) significantly increased the number of APs during windup (75.0 ± 31.9%, $n = 5$, $P < .001$ compared to control) and its efficacy (slope: Fig. 5B, middle graph; control: 3.86 ± 0.27; TGOT 5.55 ± 0.17; $n = 5$; $P < .001$). A coadministration of TGOT + dOVT did not affect the pronociceptive effects (43.1 ± 25.0%, $n = 5$, $P > .05$ compared to TGOT alone), whereas it was blocked and even reversed in the presence of AV_{1A} (Fig. 5B, right graph: −22.2 ± 10.7%, $n = 5$, $P < .001$ compared to TGOT alone). This facilitation or inhibition of this central sensitization phenomenon was also confirmed while analyzing the slope reflecting the incremental increase to generate wind up (Fig. 5, left and middle panels).

4. Discussion

In the present study, we demonstrated that low and high concentrations of TGOT and AVP in the blood are limiting or increasing spinal nociceptive processing, respectively. In our experimental conditions, antinociceptive effects were clearly mediated by OTR whereas pronociceptive effects were contributed by V_{1A}Rs. This was also true after repetitive stimulation that triggers central sensitization. These conclusions were further supported here by using a model of stress-induced analgesia associated with increased levels of OT in the blood. Blockade of OTR activity strongly limited analgesia resulting from this swim stress.

It is well established that the antinociceptive effect of OT involves several CNS structures and among them the spinal cord [4,29,36,59]. Central AVP has also been demonstrated to play a neuromodulatory role on nociception [21]. Contrary to these central effects, very few studies have focused on peripheral actions of these 2 neuropeptides on pain processing. To possibly identify any peripheral effect, the circulating concentration range of OT and AVP need to be estimated. Under basal conditions, the circulating level of OT in adult male rat are comprised in a range between 10 and 100 pg/mL, whereas AVP level is lower and comprised within 1 and 2 pg/mL [16,19]. These concentration may fluctuate slightly during night and day [11] and especially because both hormones have a short half-life (1–3 min) in the blood [15]. In the present study, the most potent analgesic dose of OT was 50 ng and this could correspond to a mean plasmatic concentration of about 3 ng/mL according to a total rat blood volume of 15 mL. According to the literature, such concentrations are likely to be reached in lactating females [1] or after stress [11,24]. Some studies have investigated the role of i.v. administration of OT but results led to contradictory conclusions. Moreover, the doses used were out of the physiologic range. Whereas analgesia was observed when OT was injected at doses of 0.2 to 2 mg/kg (~3 to 30 μg/mL) [18], Yang and colleagues [56] failed to modify nociceptive thresholds after an i.v. injection of OT at 0.1 mg/kg (~17 μg/mL). In the present study, we demonstrated that systemic AVP induced pronociceptive effects and the most effective dose was 5 ng. This could correspond to a plasmatic concentration of 300 pg/mL which may be reached during inflammatory processes [3,50]. If several studies have already demonstrated that AVP modulates nociception in humans and animal models when administered i.c.v. [2,20,26,40,53] or i.t. [34,45,46], there is, to our knowledge, no studies assessing the consequences of an intravenous injection of AVP. This study therefore provides interesting information about the possible use of these peripheral peptides as analgesics.

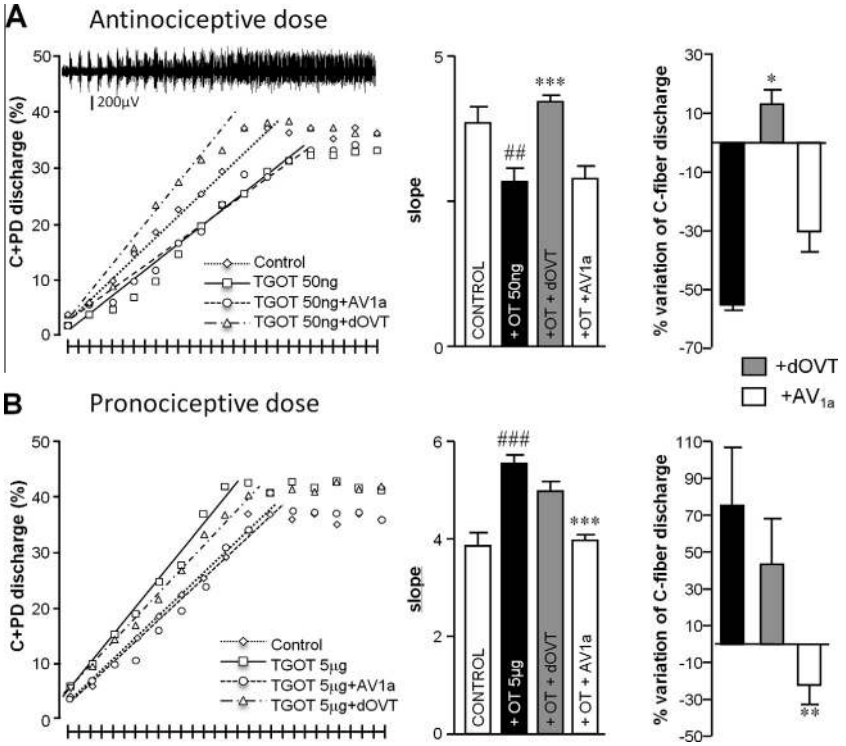

Fig. 5. Graphs illustrating the modulation of windup (representative trace shown in inset) in (A) by an antinociceptive (A) and pronociceptive (B) dose of TGOT. Effects on windup slope to reach the plateau phase for a representative neuron (left graphs) and for all neurons (middle histograms) are shown together with the variation of C-fiber discharge (right histograms) for all pharmacological conditions: TGOT alone, TGOT + dOVT (OTR antagonist, 50 µg) and TGOT + AV_{1A} (V_{1A}R antagonist, 50 µg). Statistical significance with Bonferroni post hoc tests after 1-way ANOVA is indicated as follows: control vs TGOT ($^{\#\#}P < .01$, $^{\#\#\#}P < .001$), TGOT vs TGOT + antagonists ($^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$).

Because AVP and OT, at physiological doses, do not cross the blood–brain barrier [12,34], our data suggest that these hormones could affect spinal nociceptive processing by a peripheral mechanism. This may involve an action on DRG neurons. To support this idea, Tan and coworkers [44] have demonstrated in vitro that low concentration of OT (10^{-12} to 10^{-9} M) enhanced GABA-activated currents possibly leading to the suppression of sensorispinal transmission, whereas application of OT at higher doses (>10^{-9} M) reduced the presynaptic inhibition of GABA. This biphasic effect is fully in agreement with our in vivo study. We failed to reveal any possible spinal or supraspinal contribution after intravenous injection of OT or AVP receptor agonists. This conclusion was reached because (i) the direct spinal application of dOVT was without effect on spinal pain processing indicating that no tonic hypothalamospinal release was present or modulated ($n = 12$, not shown) and (ii) spinal dOVT application never occluded the pro- or antinociceptive action of OT/AVP receptor agonists after intravenous infusion ($n = 6$, not shown). Taken together, this led us to assume that OTR in the DRG and/or at the periphery [10] are involved in the modulation of pain processing by circulating levels of OT and AVP.

In the present work, the 2 neurohormones displayed both anti- and pronociceptive effects, which we demonstrate to be related to the activation of OTR and V_{1A}R receptors, respectively. In the electrophysiological experiments, we clearly observed that the V_{1A}R antagonist reversed the pronociceptive effects induced by high doses of AVP (50 ng) or OT (5 µg). On the other hand, the OTR antagonist dOVT limited antinociception induced by systemic injections of low doses of AVP (50 pg) or OT (50 ng). A similar conclusion was reached could be drawn while using a stimulation protocol giving rise to an amplified and prolonged nociceptive AP discharge (windup) that may reflect central sensitization. This suggests that, besides the acute processing of pain messages, peripheral administration of low doses of OT may limit excitability in the nociceptive system in pathological pain states.

The modulatory role of OTR and V_{1A}R observed in in vivo anesthetized rats was further confirmed with freely moving rats using the swim stress-induced analgesia paradigm known to induce a plasmatic release of OT [54]. Using a model of knockout mice, it has recently been demonstrated that OT-induced mechanical and thermal analgesia could be observed in WT and $OTR^{-/-}$ but was only absent in V_{1A}R knockout mice [40]. This puzzling result pushes the idea that V_{1A}R, at least in mice, could account for most if not all antinociceptive effects after peripheral exogenous OT injection. Beside the difficulty to really know at which concentration OT and AVP reach their specific receptors after being injected i.p. at high doses (OT: 0.1–8 mg/kg; AVP 0.1–0.5 mg/kg), we can only propose that these differences may rely on interspecies differences (rat vs mouse). Such interspecies differences have also been documented for OT and AVP and for other peptides. An excessive neuronal uptake of OT has also been observed when OT was injected in large amounts (~100 µg/animal) [12]. This consequently may bias the interpretation of OT action, especially with regards to the opposite effects reached by different doses of neuropeptides.

In summary, our study provides novel data that better define how a single dose of OT and AVP in the blood could have anti- and pronociceptive effects. We clearly demonstrate that OTR activation is required to induce analgesia by OT and AVP. On the other hand, V_{1A}R activation is associated with hypernociception in rats. Eventually, we demonstrated the endogenous peripheral release of OT contribute to stress-induced analgesia through the activation of OTRs, possibly expressed by small-caliber DRG neurons (eg, unmyelinated C-type nociceptors) [31]. Because antinociceptive effects could also be observed after central sensitization, our results raise the question of whether these hormones could be of clinical interest for the treatment of chronic pain states.

Conflict of interest

The authors report no conflict of interest.

Acknowledgments

We thank Glenn-Marie Le Coz and Chamece-Addine Arahouani for their excellent technical assistance during the project. The following French institutions supported this work: Centre National de la Recherche Scientifique, Université de Strasbourg, Institut Universitaire de France (PP). PEJ was a postdoctoral fellow supported by a Marie Curie Actions of the European Commission (FP7-COFUND) attributed to the National Research Fund of Luxembourg.

References

[1] Amico JA, Challinor SM, Cameron JL. Pattern of oxytocin concentrations in the plasma and cerebrospinal fluid of lactating rhesus monkeys (Macaca mulatta): evidence for functionally independent oxytocinergic pathways in primates. J Clin Endocrinol Metab 1990;71:1531–5.
[2] Bodnar RJ, Wallace MM, Kordower JH, Nilaver G, Cort J, Zimmerman EA. Modulation of nociceptive thresholds by vasopressin in the Brattleboro and normal rat. Ann N Y Acad Sci 1982;394:735–9.
[3] Brackett DJ, Schaefer CF, Tompkins P, Fagraeus L, Peters LJ, Wilson MF. Evaluation of cardiac output, total peripheral vascular resistance, and plasma concentrations of vasopressin in the conscious, unrestrained rat during endotoxemia. Circ Shock 1985;17:273–84.
[4] Breton JD, Veinante P, Uhl-Bronner S, Vergnano AM, Freund-Mercier MJ, Schlichter R, Poisbeau P. Oxytocin-induced antinociception in the spinal cord is mediated by a subpopulation of glutamatergic neurons in lamina III which amplify GABAergic inhibition. Mol Pain 2008;4:19.
[5] Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994;53:55–63.
[6] Chini B, Manning M. Agonist selectivity in the oxytocin/vasopressin receptor family: new insights and challenges. Biochem Soc Trans 2007;35:737–41.
[7] Condes-Lara M, Gonzalez NM, Martinez-Lorenzana G, Delgado OL, Freund-Mercier MJ. Actions of oxytocin and interactions with glutamate on spontaneous and evoked dorsal spinal cord neuronal activities. Brain Res 2003;976:75–81.
[8] Condes-Lara M, Maie IA, Dickenson AH. Oxytocin actions on afferent evoked spinal cord neuronal activities in neuropathic but not in normal rats. Brain Res 2005;1045:124–33.
[9] Condes-Lara M, Rojas-Piloni G, Martinez-Lorenzana G, Rodriguez-Jimenez J. Paraventricular hypothalamic oxytocinergic cells responding to noxious stimulation and projecting to the spinal dorsal horn represent a homeostatic analgesic mechanism. Eur J Neurosci 2009;30:1056–63.
[10] Denda S, Takei K, Kumamoto J, Goto M, Tsutsumi M, Denda M. Oxytocin is expressed in epidermal keratinocytes and released upon stimulation with adenosine 5′-[gamma-thio]triphosphate in vitro. Exp Dermatol 2012;21:535–7.
[11] Devarajan K, Rusak B. Oxytocin levels in the plasma and cerebrospinal fluid of male rats: effects of circadian phase, light and stress. Neurosci Lett 2004;367:144–7.
[12] Ermisch A, Ruhle HJ, Landgraf R, Hess J. Blood–brain barrier and peptides. J Cereb Blood Flow Metab 1985;5:350–7.
[13] Gao L, Yu LC. Involvement of opioid receptors in the oxytocin-induced antinociception in the central nervous system of rats. Regul Pept 2004;120:53–8.
[14] Ge Y, Lundeberg T, Yu LC. Blockade effect of mu and kappa opioid antagonists on the anti-nociception induced by intra-periaqueductal grey injection of oxytocin in rats. Brain Res 2002;927:204–7.
[15] Gimpl G, Fahrenholz F. The oxytocin receptor system: structure, function, and regulation. Physiol Rev 2001;81:629–83.
[16] Hashimoto H, Hyodo S, Kawasaki M, Mera T, Chen L, Soya A, Saito T, Fujihara H, Higuchi T, Takei Y, Ueta Y. Centrally administered adrenomedullin 2 activates hypothalamic oxytocin-secreting neurons, causing elevated plasma oxytocin level in rats. Am J Physiol Endocrinol Metab 2005;289:E753–61.
[17] Howl J, Filer AD, Parslow RA, Kirk CJ, Jurzak M, Smith AI, Wheatley M. Pharmacological characterization of linear analogues of vasopressin generated by the systematic substitution of positions 1 and 6 by L-amino acids. Biochem Pharmacol 1994;47:1497–501.
[18] Kang YS, Park JH. Brain uptake and the analgesic effect of oxytocin—its usefulness as an analgesic agent. Arch Pharm Res 2000;23:391–5.
[19] Katoh A, Fujihara H, Ohbuchi T, Onaka T, Young 3rd WS, Dayanithi G, Yamasaki Y, Kawata M, Suzuki H, Otsubo H, Murphy D, Ueta Y. Specific expression of an oxytocin-enhanced cyan fluorescent protein fusion transgene in the rat hypothalamus and posterior pituitary. J Endocrinol 2011;204:275–85.
[20] Kordower JH, Sikorszky V, Bodnar RJ. Central antinociceptive effects of lysine-vasopressin and an analogue. Peptides 1982;3:613–7.
[21] Koshimizu TA, Tsujimoto G. New topics in vasopressin receptors and approach to novel drugs: vasopressin and pain perception. J Pharmacol Sci 2009;109:33–7.
[22] Leuner B, Caponiti JM, Gould E. Oxytocin stimulates adult neurogenesis even under conditions of stress and elevated glucocorticoids. Hippocampus 2011;22:861–8.
[23] Liu X, Tribollet E, Ogier R, Barberis C, Raggenbass M. Presence of functional vasopressin receptors in spinal ventral horn neurons of young rats: a morphological and electrophysiological study. Eur J Neurosci 2003;17:1833–46.
[24] Lukic D, Haldar J. Isotonic and hypertonic saline act as stressful stimuli for oxytocinergic system of the pituitary, hypothalamus and spinal cord. Life Sci 1993;53:579–84.
[25] Lundeberg T, Uvnas-Moberg K, Agren G, Bruzelius G. Anti-nociceptive effects of oxytocin in rats and mice. Neurosci Lett 1994;170:153–7.
[26] Madrazo I, Franco-Bourland RE, Leon-Meza VM, Mena I. Intraventricular somatostatin-14, arginine vasopressin, and oxytocin: analgesic effect in a patient with intractable cancer pain. Appl Neurophysiol 1987;50:427–31.
[27] Martinez-Lorenzana G, Espinosa-Lopez L, Carranza M, Aramburo C, Paz-Tres C, Rojas-Piloni G, Condes-Lara M. PVN electrical stimulation prolongs withdrawal latencies and releases oxytocin in cerebrospinal fluid, plasma, and spinal cord tissue in intact and neuropathic rats. PAIN® 2008;140:265–73.
[28] Millan MJ, Schmauss C, Millan MH, Herz A. Vasopressin and oxytocin in the rat spinal cord: analysis of their role in the control of nociception. Brain Res 1984;309:384–8.
[29] Miranda-Cardenas Y, Rojas-Piloni G, Martinez-Lorenzana G, Rodriguez-Jimenez J, Lopez-Hidalgo M, Freund-Mercier MJ, Condes-Lara M. Oxytocin and electrical stimulation of the paraventricular hypothalamic nucleus produce antinociceptive effects that are reversed by an oxytocin antagonist. PAIN® 2006;122:182–9.
[30] Mogil JS, Sorge RE, LaCroix-Fralish ML, Smith SB, Fortin A, Sotocinal SG, Ritchie J, Austin JS, Schorscher-Petcu A, Melmed K, Czerminski J, Bittong RA, Mokris JB, Neubert JK, Campbell CM, Edwards RR, Campbell JN, Crawley JN, Lariviere WR, Wallace MR, Sternberg WF, Balaban CD, Belfer I, Fillingim RB. Pain sensitivity and vasopressin analgesia are mediated by a gene–sex–environment interaction. Nat Neurosci 2011;14:1569–73.
[31] Moreno-Lopez Y, Martinez-Lorenzana G, Condés-Lara M, Rojas-Piloni G. Identification of oxytocin receptor in the dorsal horn and nociceptive dorsal root ganglion neurons. Neuropeptides 2013;47:117–23.
[32] Reeta K, Mediratta PK, Rathi N, Jain H, Chugh C, Sharma KK. Role of kappa- and delta-opioid receptors in the antinociceptive effect of oxytocin in formalin-induced pain response in mice. Regul Pept 2006;135:85–90.
[33] Reiter MK, Kremarik P, Freund-Mercier MJ, Stoeckel ME, Desaulles E, Feltz P. Localization of oxytocin binding sites in the thoracic and upper lumbar spinal cord of the adult and postnatal rat: a histoautoradiographic study. Eur J Neurosci 1994;6:98–104.
[34] Robbins ICAF. Neurohypophysial peptides in cerebrospinal fluid. In: Cross BA, Leng G, editors. The neurohypophysis: structure, function and control. Amsterdam: Elsevier; 1983. p. 129–55.
[35] Robinson DA, Wei F, Wang GD, Li P, Kim SJ, Vogt SK, Muglia LJ, Zhuo M. Oxytocin mediates stress-induced analgesia in adult mice. J Physiol 2002;540:593–606.
[36] Rojas-Piloni G, Mejia-Rodriguez R, Martinez-Lorenzana G, Condes-Lara M. Oxytocin, but not vassopressin, modulates nociceptive responses in dorsal horn neurons. Neurosci Lett 2010;476:32–5.
[37] Rossier J, Battenberg E, Pittman Q, Bayon A, Koda L, Miller R, Guillemin R, Bloom F. Hypothalamic enkephalin neurones may regulate the neurohypophysis. Nature 1979;277:653–5.
[38] Schmidt A, Audigier S, Barberis C, Jard S, Manning M, Kolodziejczyk AS, Sawyer WH. A radioiodinated linear vasopressin antagonist: a ligand with high affinity and specificity for V1a receptors. FEBS Lett 1991;282:77–81.
[39] Schoneberg T, Kostenis E, Liu J, Gudermann T, Wess J. Molecular aspects of vasopressin receptor function. Adv Exp Med Biol 1998;449:347–58.
[40] Schorscher-Petcu A, Sotocinal S, Ciura S, Dupre A, Ritchie J, Sorge RE, Crawley JN, Hu SB, Nishimori K, Young LJ, Tribollet E, Quirion R, Mogil JS. Oxytocin-induced analgesia and scratching are mediated by the vasopressin-1A receptor in the mouse. J Neurosci 2010;30:8274–84.
[41] Sofroniew MV. Morphology of vasopressin and oxytocin neurones and their central and vascular projections. Prog Brain Res 1983;60:101–14.
[42] Swanson LW, Kuypers HG. The paraventricular nucleus of the hypothalamus: cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labeling methods. J Comp Neurol 1980;194:555–70.
[43] Swanson LW, Sawchenko PE. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. Annu Rev Neurosci 1983;6:269–324.
[44] Tan ZJ, Wei JB, Li ZW, Shao M, Hu QS, Peng BW. Modulation of GABA-activated currents by oxytocin in rat dorsal root ganglion neurons. Sheng Li Xue Bao 2000;52:381–4.
[45] Thurston CL, Campbell IG, Culhane ES, Carstens E, Watkins LR. Characterization of intrathecal vasopressin-induced antinociception, scratching behavior, and motor suppression. Peptides 1992;13:17–25.
[46] Thurston CL, Culhane ES, Suberg SN, Carstens E, Watkins LR. Antinociception vs motor effects of intrathecal vasopressin as measured by four pain tests. Brain Res 1988;463:1–11.

[47] Tribollet E, Barberis C, Arsenijevic Y. Distribution of vasopressin and oxytocin receptors in the rat spinal cord: sex-related differences and effect of castration in pudendal motor nuclei. Neuroscience 1997;78:499–509.
[48] Tribollet E, Charpak S, Schmidt A, Dubois-Dauphin M, Dreifuss JJ. Appearance and transient expression of oxytocin receptors in fetal, infant, and peripubertal rat brain studied by autoradiography and electrophysiology. J Neurosci 1989;9:1764–73.
[49] Urch CE, Dickenson AH. In vivo single unit extracellular recordings from spinal cord neurones of rats. Brain Res Protocols 2003;12:26–34.
[50] Varga C, Pavo I, Lamarque D, Szepes Z, Kiss J, Karacsony G, Laszlo FA, Laszlo F. Endogenous vasopressin increases acute endotoxin shock-provoked gastrointestinal mucosal injury in the rat. Eur J Pharmacol 1998;352:257–61.
[51] Veronneau-Longueville F, Rampin O, Freund-Mercier MJ, Tang Y, Calas A, Marson L, McKenna KE, Stoeckel ME, Benoit G, Giuliano F. Oxytocinergic innervation of autonomic nuclei controlling penile erection in the rat. Neuroscience 1999;93:1437–47.
[52] Wang JW, Lundeberg T, Yu LC. Antinociceptive role of oxytocin in the nucleus raphe magnus of rats, an involvement of mu-opioid receptor. Regul Pept 2003;115:153–9.
[53] Watkins LR, Suberg SN, Thurston CL, Culhane ES. Role of spinal cord neuropeptides in pain sensitivity and analgesia: thyrotropin releasing hormone and vasopressin. Brain Res 1986;362:308–17.
[54] Wotjak CT, Ganster J, Kohl G, Holsboer F, Landgraf R, Engelmann M. Dissociated central and peripheral release of vasopressin, but not oxytocin, in response to repeated swim stress: new insights into the secretory capacities of peptidergic neurons. Neuroscience 1998;85:1209–22.
[55] Yang J, Li P, Zhang XY, Zhang J, Hao F, Pan YJ, Lu GZ, Lu L, Wang DX, Wang G, Yan FL. Arginine vasopressin in hypothalamic paraventricular nucleus is transferred to the caudate nucleus to participate in pain modulation. Peptides 2011;32:71–4.
[56] Yang J, Yang Y, Chen JM, Liu WY, Wang CH, Lin BC. Central oxytocin enhances antinociception in the rat. Peptides 2007;28:1113–9.
[57] Yang J, Yang Y, Chen JM, Xu HT, Liu WY, Lin BC. Arginine vasopressin in periaqueductal gray, which relates to antinociception, comes from hypothalamic paraventricular nucleus in the rat. Neurosci Lett 2007;412:154–8.
[58] Yang J, Yuan H, Liu W, Song C, Xu H, Wang G, Ni N, Yang D, Lin B. Arginine vasopressin in hypothalamic paraventricular nucleus is transferred to the nucleus raphe magnus to participate in pain modulation. Peptides 2009;30:1679–82.
[59] Yu SQ, Lundeberg T, Yu LC. Involvement of oxytocin in spinal antinociception in rats with inflammation. Brain Res 2003;983:13–22.