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Blockade of vasopressin receptors reduces the threshold pressure of micturition reflex in female rats

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ABSTRACT

The mechanisms involved in urinary bladder control are not fully understood, but it is well accepted that a complex central network is involved in micturition control. The micturition reflex can be modulated by direct cortical influence through facilitatory and inhibitory mechanisms. In addition, humoral mechanisms are involved in the bladder control. Vasopressin increases bladder contraction and intravesical pressure. This study sought to investigate the effect of intravenous injections of vasopressin receptor antagonists on cystometric parameters in anesthetized female rats. Isoflurane anesthetized adult female Wistar rats underwent femoral artery and vein cannulation for arterial pressure (AP) and heart rate (HR) recordings, and infusion of drugs, respectively. The bladder was also cannulated for intravesical pressure (IP) recordings and infusion of saline (10 mL/h) for cystometric evaluation. After baseline AP, HR and IP recordings, saline (vehicle, 1 mL/kg), V1a (5 μg/kg) or V2 receptor antagonist (5 μg/kg) was injected i.v. and after 25 min the cystometry was carried out. Neither saline nor V1a or V2 receptor blockade evoked any change in AP, HR and IP. Nevertheless, during cystometry, the threshold pressure of the micturition reflex was significantly reduced in rats with V1a (to 19.30 ± 2.39 mmHg) and V2 receptor blockade (to 19.88 ± 2.49 mmHg) compared to the saline group (28.85 ± 2.06 mmHg, $p = 0.014$). No difference was observed in the other cystometric parameters. Therefore, the data suggest that blockade of V1a and V2 receptors reduces the threshold pressure of the micturition reflex and does not influence other cystometric parameters in anesthetized female Wistar rats.

1. Introduction

Urinary bladder dysfunctions affect millions of people worldwide with higher prevalence in women than in men (Irwin et al., 2011). Voiding dysfunctions can cause emotional, psychological and social discomfort, and affecting well-being, with patients often suffering in silence due to the difficulty of performing various normal activities in the daily life (Kajiwara et al., 2004; Sureshkumar et al., 2009).

A complex network of neurons in the brain and spinal cord controls the urinary bladder and urethral sphincters, allowing urine to be stored and voided when socially appropriate. Contraction and relaxation of the detrusor muscle is dependent on the parasympathetic and sympathetic autonomic nervous systems, respectively. Urine storage is facilitated by distension of the bladder during filling, synchronized with contraction of the urethral sphincters. This reflex mechanism, which is integrated in the sacral level of the spinal cord (S2–S4), can respond to direct cortical influence through facilitatory and inhibitory mechanisms. As urine storage causes bladder filling, stretch receptors are stimulated, sending information to the micturition center. Consequently, parasympathetic neurons are activated, generating rhythmic contractions of the detrusor muscle and relaxation of the internal sphincter of the urethra. During voiding, contraction of the detrusor muscle of the bladder occurs synchronized with relaxation of the striated urethral sphincter (Andersson and Hedlund, 2002; de Groat, 1998; Arya and Weissbart, 2017).

Nevertheless, the onset of micturition is facilitated by the Pontine Micturition Center (PMC), whereas urinary storage is influenced by the Pontine Urine Storage Center (PUSC), which is found ventrolaterally to the PMC (de Groat, 1998). In rats, the PMC corresponds to the Barrington's nucleus (Sugaya et al., 2005), which modulates the micturition reflex. The mechanism is based on bladder pressure, which consequently

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interferes with the coordination of actions in the detrusor muscle and urethral sphincter (Sugaya et al., 2005).

The sensation or desire to urinate is triggered by sensory information from the bladder, which follows two afferent pathways. One of them depends on fibers ascending through the dorsal funicular of the spinal cord and projects into the cuneate nucleus in the medulla (Sugaya et al., 2005). The second pathway has its fibers in the spinothalamic tract, which travels through the lateral funicular of the spinal cord. Both pathways from the bladder project their fibers into the thalamus and subsequently activate sensory areas of the cerebral cortex (de Groat, 1998; Sugaya et al., 2005). The efferent pathway from the Barrington's nucleus inhibits the thoraco-lumbar sympathetic nuclei and excites the pudendal nucleus (Onuf's nucleus) in the sacral region. In the micturition reflex, the increased afferent firing from stretch receptors stimulated by bladder filling produces firing in the sacral parasympathetic pathways and inhibition of sympathetic and somatic pathways. The expulsion phase depends on an initial relaxation of the urethral sphincter followed by a contraction of the bladder, an increase in bladder pressure, and flow of urine. Relaxation of the urethral outlet is mediated by activation of a parasympathetic reflex pathway to the urethra, which triggers the release of NO, an inhibitory transmitter, as well as by removal of adrenergic and somatic excitatory inputs to the urethra (de Groat et al., 2015).

Although classical pathways that control the micturition have been demonstrated, Cafarchio et al. (2016) have shown that activation of cholinergic neurons in the medulla increases intravesical pressure through release of vasopressin into the plasma, which in turn, activates vasopressin receptors in the urinary bladder.

Previous *in vitro* studies have shown that vasopressin at concentrations above 10^{-5} M cause contraction of the urinary bladder (Crankshaw, 1989; Holmquist et al., 1991; Berggren et al., 1993). Vasopressin also increased intravesical pressure in anesthetized rats in a dose-dependent manner and both gene and protein expression for all subtypes of vasopressin receptors (V1a, V1b and V2) have been found in the bladder by qPCR and Western Blotting, respectively (Cafarchio et al., 2018).

The current pharmacological therapies for treatment of underactive bladder (Ladi-Seyedian et al., 2018) cause several side effects and the better understanding of the mechanisms involved in urinary bladder control is highly relevant for the development of novel therapeutic approaches.

Even though vasopressin increases intravesical pressure *in vitro* and *in vivo*, no previous study has evaluated whether the blockade of vasopressin receptors affects or not the micturition reflex. This study was focused at investigating the effect of intravenous injections of V1a and V2 vasopressin receptor antagonists on cystometric parameters in anesthetized female rats.

2. Materials and methods

2.1. Animals

Female Wistar rats (~250–300 g, 14-16 weeks-old, N = 19) provided by the Animal Facility of the Faculdade de Medicina do ABC/Centro Universitario FMABC, Brazil were used. The animals were housed in plastic cages (4 rats/cage) with standard chow pellets and tap water *ad libitum*, in an air-conditioned room (20–24 °C) with a 12:12-h light-dark cycle. The humidity of the animal room was maintained at ~70%. All procedures were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and were approved by the Animal Ethics Committee of the Faculdade de Medicina do ABC (protocol number 11/2018).

3. Cystometry

Rats were anesthetized with 2% isoflurane in 100% O_2, as according to previous studies this anesthetic does not affect the urinary bladder reflex and smooth muscle contraction (Hyun et al., 2009; Park et al., 2010; Cafarchio et al., 2016, 2018; Chang and Hayton, 2008), and submitted to bladder cannulation through the insertion of a polyethylene tube (PE-50 connected to PE-10, Clay Adams, NJ) for intravesical pressure (IP) recording in a data acquisition system data (PowerLab 16 SP, ADInstruments, Castle Hill, AU) and a second polyethylene tube (PE-50 connected to PE-10, Clay Adams, NJ) for cystometry. Both polyethylene tubes were filled with saline and inserted at the top of the bladder. A small drop of tissue glue was used to fix the catheters on the bladder wall. The urethra outlet was not ligated in order to permit bladder voiding if necessary. The polyethylene tube inserted into the urinary bladder was connected to a 3-way tap to allow the bladder to be filled with saline or emptied when necessary, and a baseline intravesical pressure (IP) value was set at ~10 mmHg by saline infusion or urine withdrawal through the catheter inserted into the urinary bladder.

Saline infusion was performed at a rate of 10 mL/h (Abolhasanpour et al., 2020; Park et al., 2010 and Uvin et al., 2012) using a pump (Insight, Ribeirão Preto, SP, Brazil) and the intravesical pressure was concomitantly recorded through the second polyethylene tube inserted in the urinary bladder. The following parameters were analyzed in the cystometry: threshold pressure (TP, pressure at initiation of voiding contraction), maximal intravesical pressure (IVP, maximum pressure during micturition), basal intravesical pressure (lowest intravesical pressure after voiding), voiding volume (volume voided per micturition), and threshold for the onset of premicturition contractions (TOPC). Bladder capacity (infusion rate X interval between contractions) and bladder compliance [bladder capacity/(threshold pressure - basal intravesical pressure)] were calculated (Chang and Hayton, 2008; Andersson et al., 2011; Flores et al., 2018). The latency to achieve the IVP after the onset of saline infusion (LIVP) and the latency for the onset of premicturition contractions (LTOPC) were also evaluated (Fig. 1).

3.1. Cannulation of the femoral artery and vein

Rats anesthetized with isoflurane 2% in 100% O_2 underwent cannulation of the femoral artery and vein through insertion of polyethylene tubings (PE-50 connected to PE-10, Clay Adams, NJ, USA) for pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) recordings in a data acquisition system (PowerLab 16 SP, ADInstruments), and for intravenous drug administration, respectively.

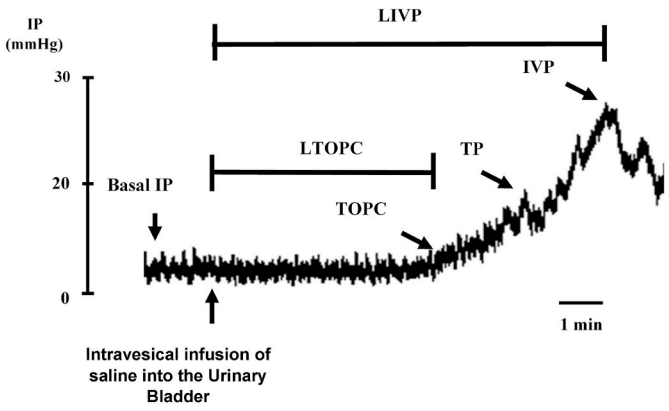

Fig. 1. Representative recording of cystometric parameters. Abbreviations: IP - intravesical pressure, TOPC - threshold for the onset of premicturition contractions, TP - threshold pressure, IVP - maximum intravesical pressure, LTOP - latency for the threshold for the onset of premicturition contractions after starting the intravenous infusion of saline into the bladder, LIVP- latency for the maximum intravesical pressure after starting the intravenous infusion of saline into the bladder.

3.2. Drugs

- Anesthetics: isoflurane (BioChimico, Itatiaia, RJ, Brazil), and sodium thiopental (Cristalia, Itapira, SP, Brazil).
- [β-mercapto-β, β-cyclopentamethylene-propionyl1, O–Et-Tyr2, Val4-Arg8] - Vasopressin (Manning compound, V1a receptor antagonist, Sigma Aldrich, St. Louis, MO, USA)
- [Adamantaneacetyl1, O–Et-D-Tyr2, Val4, Aminobutyryl6, Arg8,9] -Vasopressin (V2 receptor antagonist, Sigma Aldrich, St. Louis, MO, USA).

3.3. Experimental protocol

3.3.1. Evaluation of V1a or V2 receptor blockade on cardiovascular and cystometric parameters in female rats

Rats were anesthetized with isoflurane and submitted to catheterization of the femoral artery and vein, and cannulation of the urinary bladder as described above. After baseline recordings of PAP, MAP, HR and IP for 15 min, saline (1 mL/kg of b.w., vehicle), V1a receptor antagonist (5 μg/kg of b.w.) or V2 receptor antagonist (5 μg/kg of b.w.) was randomly administrated in a bolus injection. The V1a and V2 receptor antagonist solution was prepared in a concentration of 10 μg/mL. Twenty-five minutes after injection, the cystometry was started and all the parameters were recorded for 15 min. At the end of the experiments, animals were euthanized with sodium thiopental (100 mg/kg, i.v.).

3.4. Statistics

A Komolgorov-Smirnov test for normality was used to verify that the data follow a normal distribution, and then they were expressed as mean ± S.E.M. Data were submitted to one-way ANOVA for comparisons of the variables recorded among groups (saline, V1a and V2 receptor antagonist) followed by Tukey posttest. Paired Student's *t*-test was used to compare MAP and HR values before (baseline) and after saline, V1a or V2 receptor antagonist within each group. Statistical analyses were conducted using the statistical software package Sigma Stat 3.5. Significance level was set at $P < 0.05$.

4. Results

4.1. Effect of V1a or V2 receptor blockade on cardiovascular and cystometric parameters in female rats

As shown in Table 1, no significant changes were observed in MAP and HR before i.v. administration of saline (baseline, N = 7) compared to 25 min after i.v. saline injection ($p > 0.05$). In the other group of rats (N = 6), at baseline (before i.v. V1a receptor antagonist) and at 25 min after i.v. V1a receptor antagonist, no significant changes were observed in MAP and HR ($p > 0.05$). Similarly, in the group before (baseline) and after i.v. V2 receptor antagonist (N = 6), no significant differences were found in MAP and HR ($p > 0.05$). The 3 groups (saline, V1a and V2 receptor antagonists) showed no differences in baseline MAP and HR and also after the intravenous injections ($p > 0.05$). In addition, the IP at 25 min after i.v. saline or V1a or V2 receptor antagonist injection showed no difference compared to the respective IP baseline ($p > 0.05$) (Table 2).

However, during the cystometric evaluation, we observed that the threshold pressure was significantly reduced in the groups with V1a (19.30 ± 2.39 mmHg) and V2 (19.88 ± 2.49 mmHg) receptor blockade compared to the saline group (28.85 ± 2.06 mmHg, $p = 0.014$, Figs. 2 and 3). Despite that, the IVP achieved was not significantly different between the groups (31.66 ± 2.05 mmHg saline, 29.65 ± 2.66 mmHg V1a receptor antagonist, and 27.98 ± 4.35 mmHg V2 receptor antagonist) ($p > 0.05$).

The basal intravesical pressure (lowest intravesical pressure after voiding) and voiding volume were unaltered in rats administrated with V1a (10.53 ± 1.28 mmHg, 1.14 ± 0.27 mL) or V2 (13.43 ± 2.61 mmHg, 1.07 ± 0.12 mL) receptor antagonist compared to saline (13.41 ± 1.62 mmHg, 1.44 ± 0.16 mL) ($p > 0.05$).

The TOPC was not different among the rats of saline (10.90 ± 0.67 mmHg), V1a (10.87 ± 1.43 mmHg) and V2 receptor antagonist (9.85 ± 0.67 mmHg) groups ($p > 0.05$).

The bladder capacity was also not affected by blockade of V1a (1.84 ± 0.47 mL) or V2 receptors (2.44 ± 0.50 mL) compared to saline (2.52 ± 0.16 mL) ($p > 0.05$).

The bladder compliance was also not different comparing the rats with V1a (0.19 ± 0.09 mL) and V2 receptor blockade (0.44 ± 0.12 mL) with saline group (0.25 ± 0.09 mL) ($p > 0.05$).

The LIVP was similar among animals which received saline (9.02 ± 0.99 min), V1a (7.15 ± 1.67 min) and (6.70 ± 0.77 min) ($p > 0.05$). No difference was observed in the LTOPC of rats with V1a (5.33 ± 0.79 min) and V2 receptor blockade (4.08 ± 0.65 min) compared to saline group (4.43 ± 0.46 min) ($p > 0.05$).

5. Discussion

The findings of this study demonstrated that blockade of V1a or V2 receptors reduced the threshold pressure compared to saline during cystometric evaluation. Despite this effect, neither the threshold for the onset of premicturition contractions, nor any other the cystometric parameters studied were affected by injections of V1a and V2 receptor antagonists. Thus, these data suggest that vasopressin is important for the maintenance of a normal micturition reflex threshold, but that it does not affect the maximum intravesical pressure, achieved at the onset of bladder voiding. The similarity of bladder capacity and bladder compliance values were consistent with the voiding volumes measured in the groups studied.

The doses of V1a and V2 receptor antagonists (5 μg/kg of b.w.) used in the current study were unable to induce changes in baseline intravesical pressure and cardiovascular parameters. These findings differ from that of Cafarchio et al. (2018) where the antagonist used at the dose of 10 μg/kg of b.w. evoked a decrease in intravesical pressure. Indeed, in the study of Cafarchio et al. (2018), the dose of V1a and V2 receptor antagonists were two folds higher, which underpins the difference in results compared to the current study. In addition, in a pilot

Table 1
Mean arterial pressure (MAP, mmHg) and heart rate (HR, bpm) at baseline and 25 min after i.v. saline (vehicle, 1 mL/kg of b.w.) or V1a receptor antagonist (5 μg/kg of b.w.) or V2 receptor antagonist (5 μg/kg of b.w) injections. Data are as mean ± SEM.

Groups	Baseline MAP	MAP at 25 min after injections	Baseline HR	HR at 25 min after injections
Saline (N = 7)	114 ± 5	115 ± 4	362 ± 19	365 ± 13
V1a receptor antagonist (N = 6)	120 ± 2	117 ± 3	327 ± 26	315 ± 36
V2 receptor antagonist (N = 6)	110 ± 3	107 ± 6	360 ± 14	339 ± 16

Data are as mean ± SEM.

Table 2
Intravesical pressure (IP, mmHg) at baseline and 25 min after i.v. saline (vehicle, 1 mL/kg of b.w.) or V1a receptor antagonist (5 μg/kg of b.w.) or V2 receptor antagonist (5 μg/kg of b.w) injections.

Groups	Baseline IP	IP at 25 min after injections
Saline (N = 7)	9.00 ± 0.65	8.94 ± 0.73
V1a receptor antagonist (N = 6)	7.97 ± 0.57	8.27 ± 0.69
V2 receptor antagonist (N = 6)	8.27 ± 0.91	7.98 ± 0.70

Data are presented as mean ± SEM.

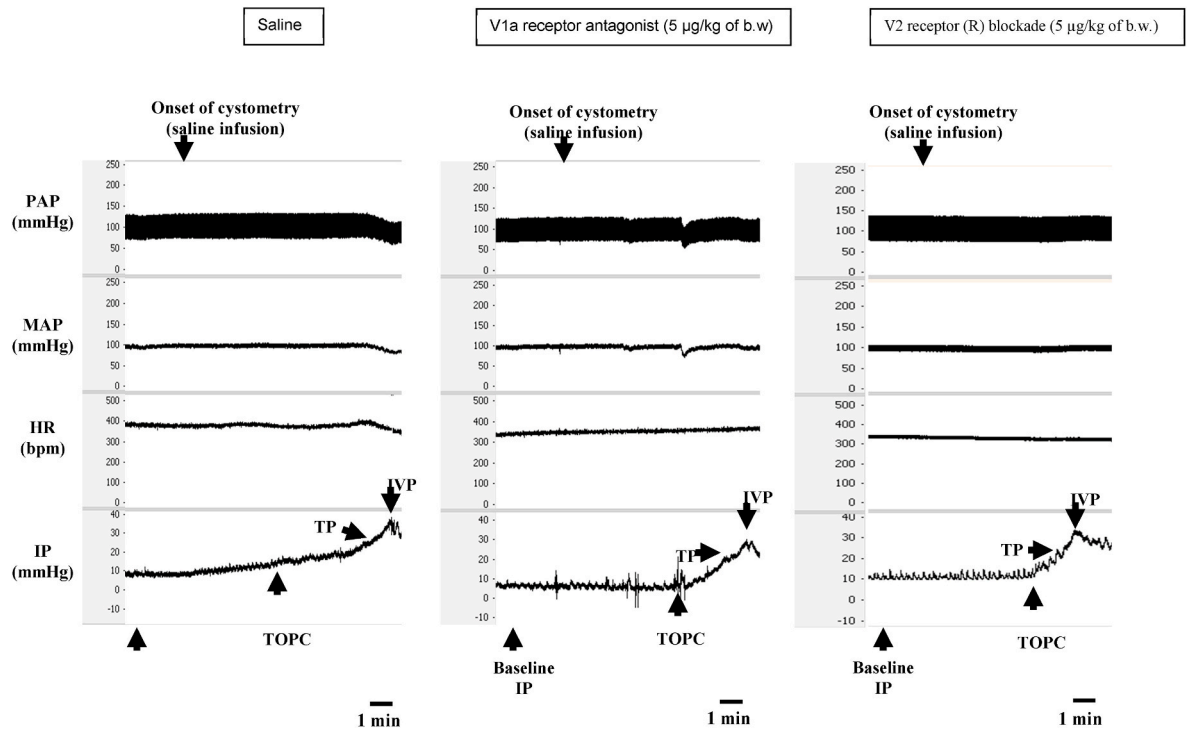

Fig. 2. Tracings showing the effects of intravenous injection of saline, V1a receptor antagonist (5 μg/kg of b.w) or V2 receptor (R) blockade (5 μg/kg of b.w.) on pulsatile arterial pressure (PAP, mmHg), mean arterial pressure (MAP, mmHg), heart rate (bpm), and intravesical pressure (IP, mmHg) during cystometric evaluation in female Wistar rats.

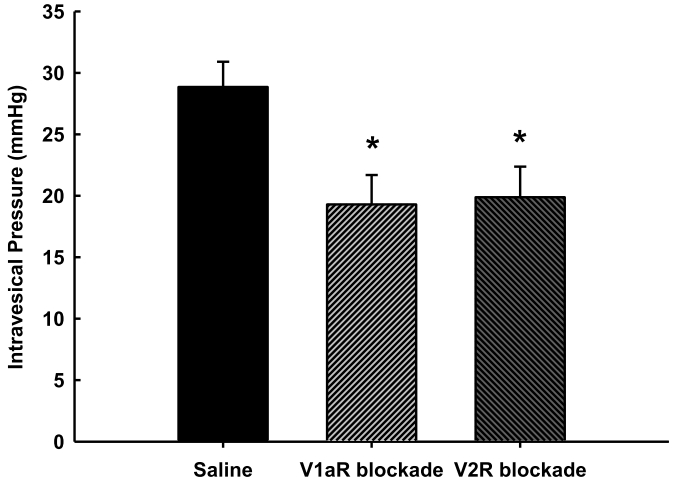

Fig. 3. Intravesical pressure (IP, mmHg) showing the threshold pressure during cystometric evaluation in rats after 25 min after intravenous injection of saline (vehicle, N = 7) or V1a (5 μg/kg of b.w., N = 6) or V2 receptor (R) blockade (5 μg/kg of b.w., N = 6) in female Wistar rats. (*P < 0.05).

experiment (unpublished data), we have evaluated if the dose of 5 μg/kg of b.w. (i.v.) of V1a and V2 receptor antagonists would not change the intravesical pressure. That dose also attenuated the increase in intravesical pressure evoked by vasopressin at the dose of 0.5 ng/kg of b.w. The notion that vasopressin maintains a normal threshold of the micturition reflex in female Wistar rats is strengthened by the fact that the baseline intravesical pressure started at the same level in all three groups of this study, however the threshold pressure of micturition reflex was only reduced in the groups which received the infusions of V1a and V2 receptor antagonists.

Vasopressin receptors are localized in the bladder, and plasma vasopressin or vasopressin infusion increases the intravesical pressure in a dose-dependent manner as previously shown by Cafarchio et al. (2016, 2018). *In vitro* studies by Berggren et al. (1993) have demonstrated that vasopressin has no effect on nerve-mediated contractile responses in the bladder. Studies of Cafarchio et al. (2018) have demonstrated by qPCR and Western Blotting that V1a and V2 receptors are expressed in the urinary bladder. Thereby, in the current study, the effect of V1a and V2 antagonists on the threshold pressure should be likely dependent on binding in the vasopressin receptors present in the bladder instead of due to action in the innervation that controls the bladder contraction.

On the other hand, evidence has shown that vasopressin can be locally synthetized in the bladder (Berggren et al., 1993). The intravesical obstruction outlet transiently decreases the immunoreactive vasopressin concentration in the bladder, although the total amount of vasopressin per bladder increases after several weeks of outlet obstruction as consequence of the increase in the bladder weight (Berggren et al., 1993). In the present study, we cannot state whether the vasopressin receptor antagonists are blocking the action of plasma vasopressin or the vasopressin locally synthetized in the bladder, which could perform a paracrine action in the bladder cells, in order to play a role in the threshold pressure of micturition.

Previous studies in female Sprague-Dawley rats have shown that isoflurane reduces the frequency of bursts, firing frequency, and amplitude of external urethral sphincter (EUS) electromyography (EMG) activity during voiding as well as the EUS EMG amplitude during the bladder filling phase compared to urethane (Chang and Hayton, 2008). Thus, it has been suggested that anesthetic properties should be taken into consideration during the interpretation of urodynamic recordings in rodent models. In the current study, the three groups of rats underwent the same anesthetic protocol. This indicates that under isoflurane anesthesia the vasopressin action on the V1a or V2 receptors in the bladder is required for a normal threshold of the micturition reflex, which needs to be confirmed using other anesthetic agents such as urethane, which is a limitation of this study. The above described differences in the cystometry reported by Chang and Hayton (2008) were

observed in Sprague-Dawley rats, which is a strain different from that used in the current study (Wistar rats). It is still unknown whether Wistar rats also show differences in the cystometric parameters upon different anesthetic protocols, which requires further investigation.

According to Ladi-Seyedian et al. (2018), patients with underactive bladder do not have an effective pharmacological treatment and the findings of the current study open the perspective for another therapeutic approach. Nevertheless, further studies are still necessary to proof the efficacy and security for use in humans.

6. Conclusion

Our data suggest that blockade of vasopressin receptors (V1a and V2) reduces the threshold pressure of the micturition reflex and does not influence other cystometric parameters in anesthetized female Wistar rats.

Compliance with ethical standards

The authors declared no conflict of interest.

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Author contributions

All authors of this study have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

CRediT authorship contribution statement

Luciana S.S. Neri: acquisition, analysis, interpretation of data, and drafting of the work. Rodrigo P. de Carvalho: acquisition, and analysis of data. Sergio A. Daiuto: acquisition, and analysis of data. Bárbara do Vale: acquisition, and analysis of data. Eduardo M. Cafarchio: analysis, interpretation of data, and drafting of the work. Patrik Aronsson: conception, interpretation on data, and revising the manuscript. Monica A. Sato: conception, analysis, interpretation of data, funding acquisition, drafting of the work.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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