Video Article

The Use of Cystometry in Small Rodents: A Study of Bladder Chemosensation

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Abstract

The lower urinary tract (LUT) functions as a dynamic reservoir that is able to store urine and to efficiently expel it at a convenient time. While storing urine, however, the bladder is exposed for prolonged periods to waste products. By acting as a tight barrier, the epithelial lining of the LUT, the urothelium, avoids re-absorption of harmful substances. Moreover, noxious chemicals stimulate the bladder's nociceptive innervation and initiate voiding contractions that expel the bladder's contents. Interestingly, the bladder's sensitivity to noxious chemicals has been used successfully in clinical practice, by intravesically infusing the TRPV1 agonist capsaicin to treat neurogenic bladder overactivity[1]. This underscores the advantage of viewing the bladder as a chemosensory organ and prompts for further clinical research. However, ethical issues severely limit the possibilities to perform, in human subjects, the invasive measurements that are necessary to unravel the molecular bases of LUT clinical pharmacology. A way to overcome this limitation is the use of several animal models[2]. Here we describe the implementation of cystometry in mice and rats, a technique that allows measuring the intravesical pressure in conditions of controlled bladder perfusion.

After laparotomy, a catheter is implanted in the bladder dome and tunneled subcutaneously to the interscapular region. Then the bladder can be filled at a controlled rate, while the urethra is left free for micturition. During the repetitive cycles of filling and voiding, intravesical pressure can be measured via the implanted catheter. As such, the pressure changes can be quantified and analyzed. Moreover, simultaneous measurement of the voided volume allows distinguishing voiding contractions from non-voiding contractions[3].

Importantly, due to the differences in micturition control between rodents and humans, cystometric measurements in these animals have only limited translational value[4]. Nevertheless, they are quite instrumental in the study of bladder pathophysiology and pharmacology in experimental pre-clinical settings. Recent research using this technique has revealed the key role of novel molecular players in the mechano- and chemo-sensory properties of the bladder.

Video Link

The video component of this article can be found at https://www.jove.com/video/3869/

Protocol

1. Laboratory Animals

1. Animals (mice, rats) are housed in a specialized animal facility with a 12-hr light-dark cycle and *ad libitum* access to water and standard food pellets. Both age and sex of the animals are important parameters that should be standardized according to the needs. We typically perform cystometry in 10 - 12 week old female animals[5,6].
2. All animal experiments are carried out in accordance with the European Union Community Council guidelines and approved by the local ethics committee.

2. Anesthesia

1. Isoflurane anesthesia is used for performing small surgical procedures, since it is easy to dose due to its short half-life. Appropriate waste gas scavenging is necessary when using isoflurane.
    a. Animals are placed in a closed box, gassed with isoflurane 5% in pure oxygen (1 l/min).
    b. After induction, anesthesia at the appropriate level is maintained by a small mask (a cut 10 ml syringe) by 0.8 - 1 l/min oxygen with 1 - 1.5% of isoflurane.

2. For mice, urethane anesthesia is used during functional recordings (cfr. Infra). Urethane anesthesia is used during functional recordings because, in contrast to most other anesthetics, the micturition reflex is not suppressed by this anesthetic agent[4]. Urethane 1.2 g/kg body weight is administered subcutaneously (s.c.) 60 min before the start of the recordings. If necessary, the dose of urethane needed to obtain proper anesthesia can be titrated by administering additional doses (0.1 g/kg) subcutaneously. It should be noted, however, that additional anesthesia may alter the voiding efficiency and consequently the micturition frequency[7,8]. Thus, the cystometric recordings should be closely monitored and discarded whenever significant deviations from the control behavior are observed. Anesthetics should not be administered while test compounds are applied to avoid misinterpretation of the data.

3. Surgical Procedure - Bladder Catheter Implantation

1. Surgical instruments are sterilized in an autoclave prior to use. Shave the abdomen of the animal, disinfect with ethanol 70% and perform a low midline laparotomy to expose the bladder.
2. Under a surgical microscope, place a purse-string suture in the bladder dome using a non-absorbable, monofilament suture (size 6-0).
3. Perform a small cystostomy (we preferentially use a 18 G needle) inside the purse string and insert a PE 50 polyethylene catheter, with a small cuff at the end (obtained by carefully heating the tubing) through this hole. In our hands, thinner tubes (PE 10) have a higher and more variable resistance. The tubing can be disinfected by putting it in an ethanol 70% solution or sterilized in an autoclave. Before implantation, the tubing should be flushed with saline.
4. Secure the purse string around the tube with a surgeon's knot. Pull the catheter gently outwards until the bell-shaped tip is positioned right under the suture. Push a piece of 18 G catheter towards the suture to prevent leaking.
5. Infuse a small volume of saline (100 - 200 µl) gently to check for leaks. If leaks are present, an additional suture should be placed.
6. Close the abdominal muscles using monofilament sutures, leaving a passage for the catheter in the upper part of the incision.
7. Tunnel the tube to the interscapular region using a hollow metal rod, thereby preventing the animals to bite the tube during the experiment.
8. The skin is closed using non-absorbable, monofilament sutures. The implanted catheter is flushed with saline to check its permeability.
9. Before the animals awaken, analgesics are given subcutaneously (buprenorphine, 0.05 mg/kg).

4. Setup and Cystometry

1. Depending on the experimental question (and the species used) cystometry is performed in awake (restraint or unrestraint) or urethane-anesthetized animals. Whereas cystometry is perfectly feasible in conscious rats, which tend to remain calm in a restrained environment, we usually perform cystometry under urethane anesthesia (1.2 mg/kg, subcutaneously) when using mice[3,5,6,9].
2. In anesthetized mice, body temperature is constantly monitored and kept at 36.5 °C ± 0.2 °C by means of a temperature probe and a heating lamp.
3. The set-up is calibrated before every experiment according to the manufacturers' instructions.
4. The implanted catheter is connected by a luer stub to a 3-way tap, which is connected to the pressure transducer (Edwards Lifesciences) on one side and an infusion pump on the other (Harvard Apparatus). The pressure transducer is connected via an amplifier (78534c monitor, Hewlett Packard) to the data acquisition system (DI-730-USB, Dataq instruments) and a computer. Windaq/Lite software can be used for recording.
5. The infusion pump is started, enabling the infusion of saline or PBS at, for example, 100 µl/min (in rats) or 20 µl/min (in mice). As such the bladder will be filled at a constant rate, while the intravesical pressure is recorded.
6. **Figure 5** depicts a typical pressure trace: during fluid infusion, there is a slow pressure buildup in the bladder, until a certain volume/pressure is reached, the micturition threshold. Once this threshold is reached, the bladder will contract and subsequently the urinary sphincter will open, allowing the passage of urine through the urethra. As such, the bladder is emptied, the contraction will stop and the pressure will drop again to the "basal" level.
7. As the rat is placed in a metabolic cage and above a digital balance, the voided volume can be measured simultaneously, giving information about the voided volume. Due to the small voided volumes in mice, it can be very challenging using this system. Therefore, voided volume is determined using small filter papers weighed before and after voiding.
8. Typically, we let the system equilibrate for 30 min, followed by a recording period of 30 min. Then, drugs can be administered systemically or intravesically and another 30 min are recorded.
9. Recorded data can be exported as .csv files that can be imported into a specialized software for quantitative analysis. We typically use Origin (OriginLab Corporation, MA, USA).
10. After experiments, animals are sacrificed by cervical dislocation (mice) or CO_2 intoxication (rats).

5. Representative Results

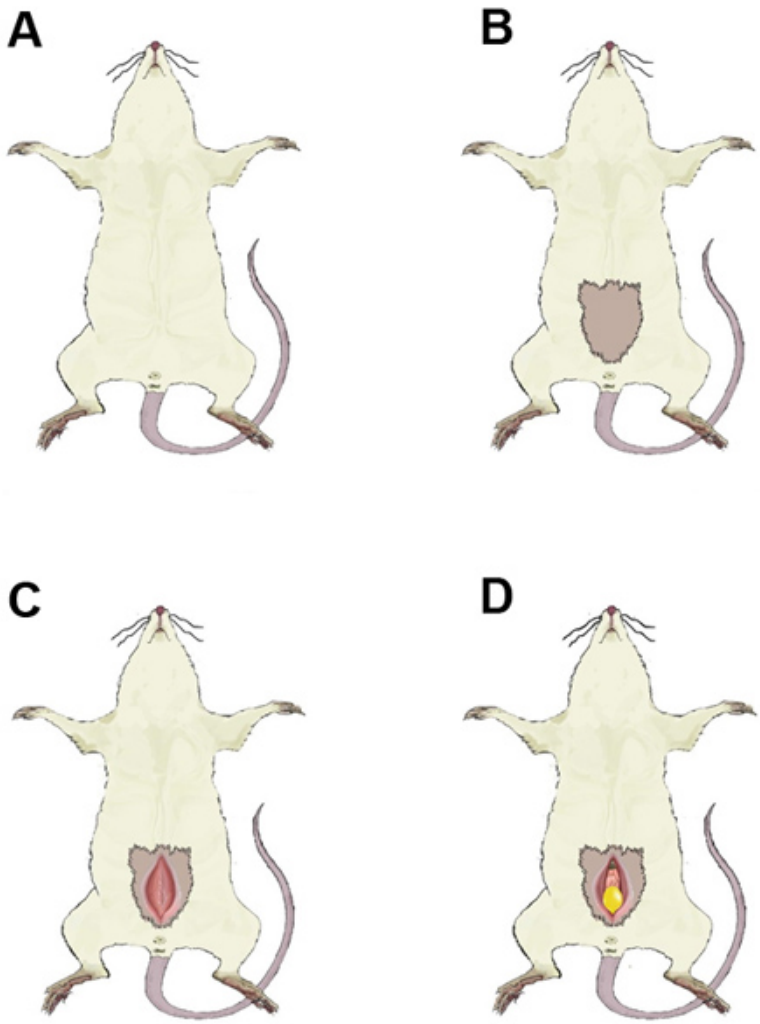

Figure 1. Laparotomy overview. A) Place the rat in the supine position. B) Shave and antiseptically prepare the surgical site. C) Incision of the skin. D) Incision of the abdominal muscles, and bladder exposure.

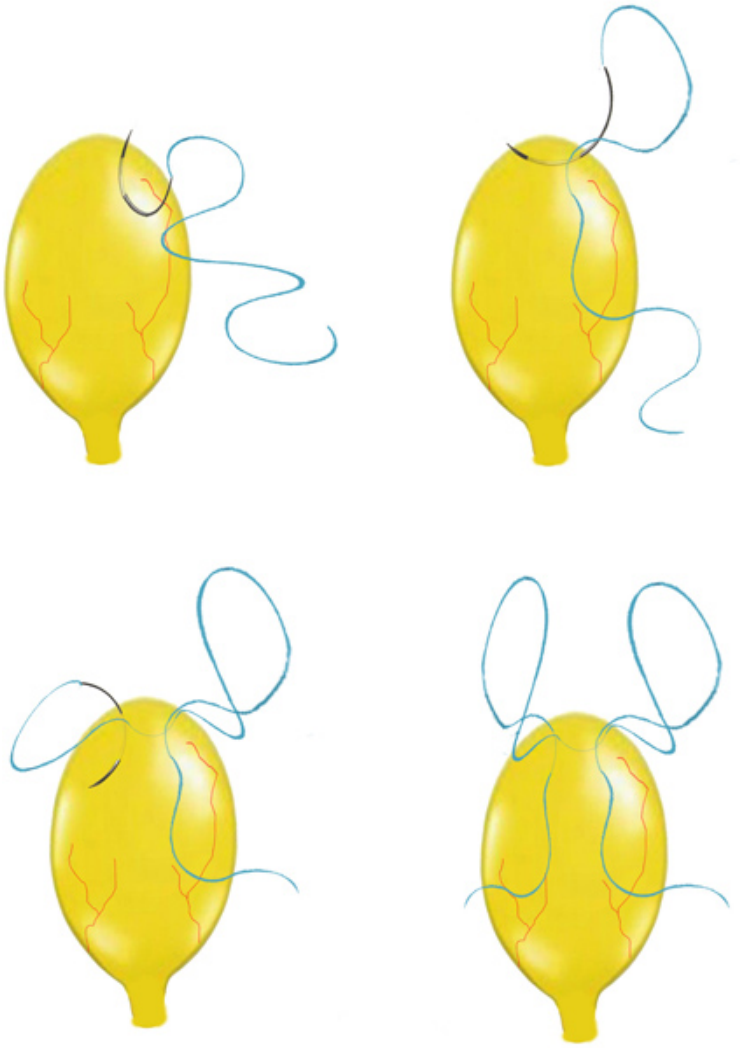

Figure 2. A purse-string suture.

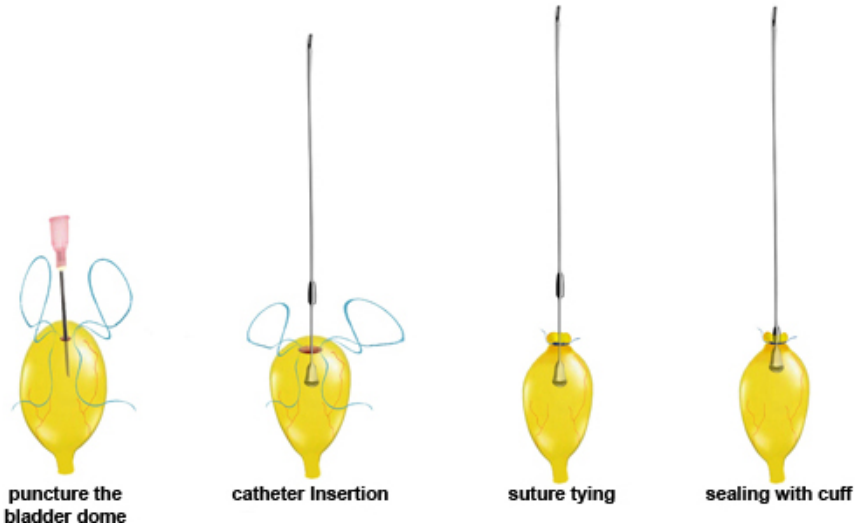

Figure 3. Catheter implantation.

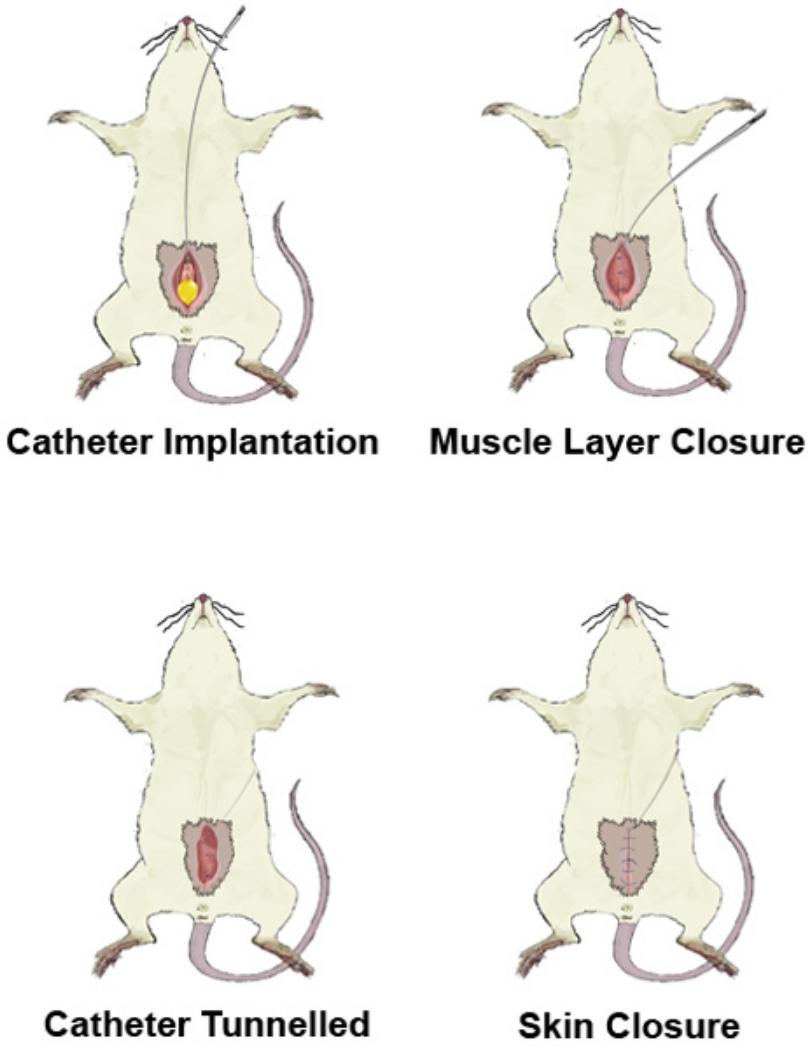

Figure 4. Tunnelling of the catheter.

Examples of pressure measurements obtained during intravesical perfusion of saline in a conscious rat and an anesthetized mouse are shown in **Figure 5**. Multiple parameters can be extracted from the pressure signal (*e.g.* the intercontractile interval, the baseline pressure and the threshold pressure). For comprehensive descriptions of these parameters, please see Andersson *et al.* (ref 4) and Yoshiyama *et al.* (ref 10).

We have recently used cystometry to identify the molecular targets of mustard oil (MO), a highly reactive compound that has been long used in experimental models of inflammation and hyperalgesia of visceral organs such as the urinary bladder[11,12]. Intravesical infusion of 10 mM MO induced a strong increase in the voiding frequency (decrease in the intercontractile interval) in wild type mice (**Figure 6A, B**) and a decrease of the voided volume[6]. Interestingly, MO induced similar changes in mice deficient of the MO receptor TRPA1. In contrast, MO induced much weaker changes in cystometric parameters in *Trpv1* KO mice than in WT mice and was without any effect in *Trpa1/Trpv1* KO mice. Together with measurements of the release of the Calcitonin Gene Related Peptide (CGRP)[6], these data indicate demonstrate that TRPV1 may play a key role in visceral irritation induced by MO.

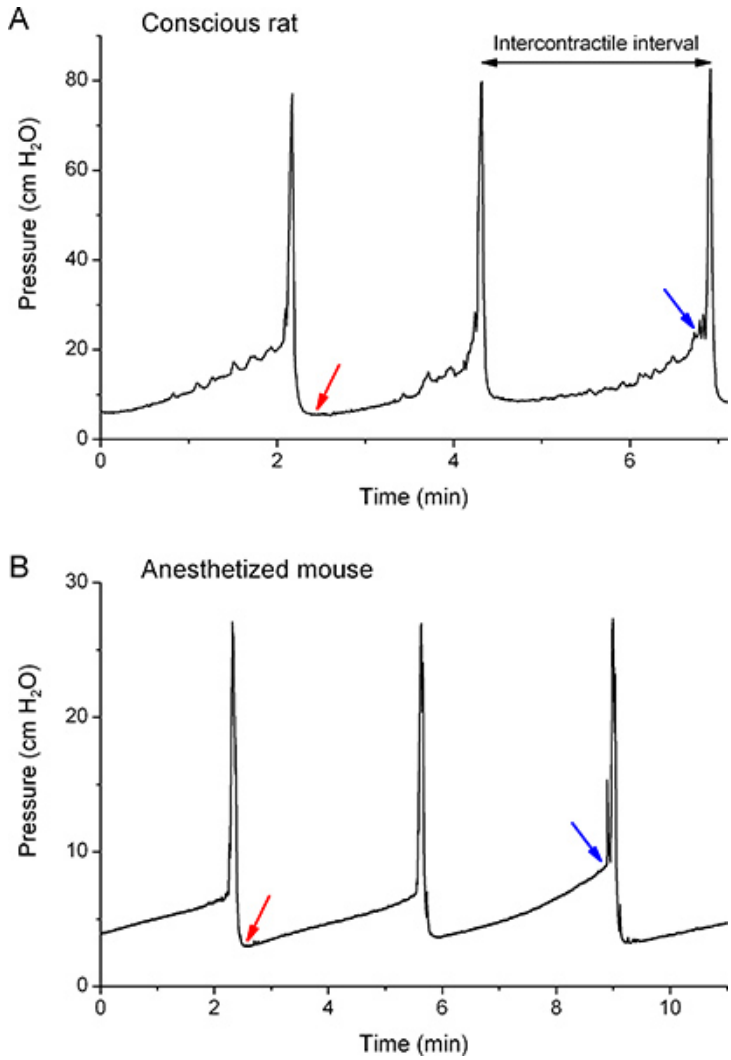

Figure 5. Representative traces of intravesical pressure recorded in a conscious female rat (A) and in an anesthetized female mouse (B). The lowest pressure is defined as the "baseline pressure" (red arrows). The pressure at the end of the filling phase is marked with blue arrows. The volume of fluid infused between these points, divided by the pressure difference, allows calculation of the compliance of the bladder wall (compliance = infused volume/(threshold pressure - baseline pressure). The "intercontractile interval" (ICI) is the time between two voiding contractions.

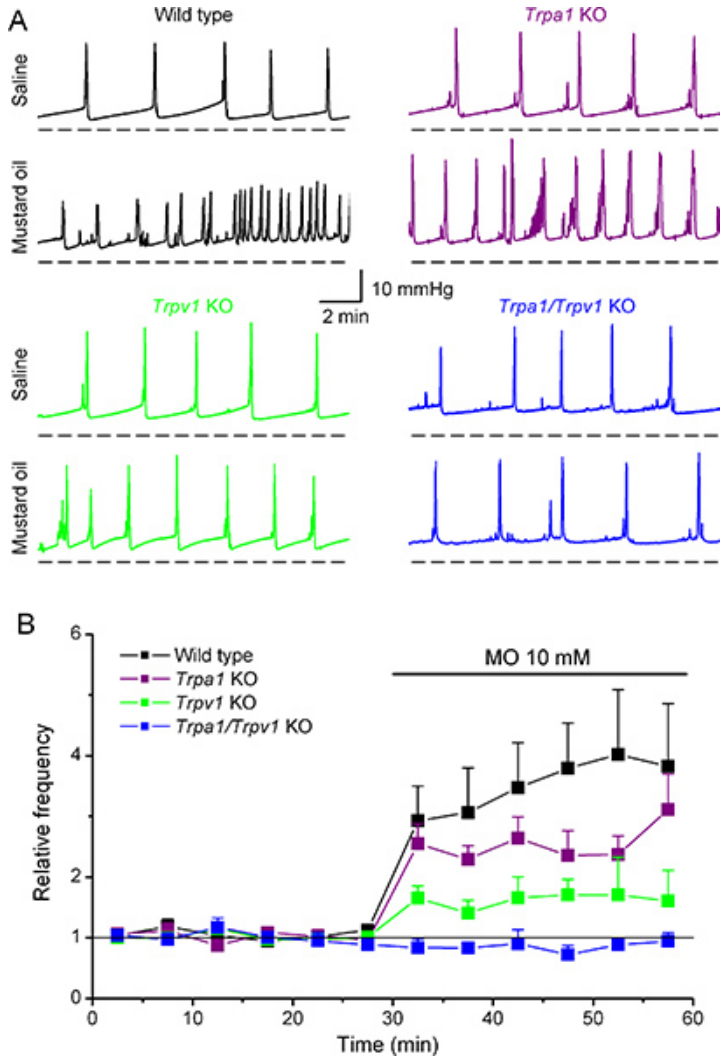

Figure 6. Effects of intravesical application of mustard oil on the cystometry pattern in wild type and *Trpa1*, *Trpv1* and *Trpa1/Trpv1* knockout mice. (A) Representative examples of intravesical pressure changes recorded in WT, *Trpa1* KO, *Trpv1* KO and *Trpa1/Trpv1* KO mice in response to infusion of saline and 10 mM MO. (B) Time course of the average instantaneous voiding frequency before and during intravesical infusion of MO. For all mice, the data were normalized to the average frequency obtained during saline infusion. These data is adapted from Everaerts *et al.* (ref 6), with permission from Elsevier.

Discussion

The cystometry technique presented here allows performing *in vivo* measurements of bladder function in animal models. Rats are probably the most used animal model. Mice are more difficult to handle, but offer the advantage of using genetically manipulated animals. Because of the technical difficulty of using conscious mice, which tend to be very active resulting in loosening of the implanted catheter and changes in the intra-abdominal pressures that may influence the intravesical pressure, we advise to keep them anesthetized with urethane during the full cystometry protocol. Of course, the benefits of having sedated mice, should be weighed against the effects of the anesthetics. As such, urethane can have a significant influence on post-voiding residual volume[7,8]. We therefore wait until we have a stable degree of anesthesia, before we start our recordings and interventions.

Researchers should consider the differences between male and female rodent micturition[13]. Also, the age of the animals is of great importance. We perform cystometry in animals that are 10 - 12 weeks old. All the experiments should be performed in a calm environment, especially when using conscious animals. A number of infusion rates have been described for our animal models. We typically use infusion rates of 20 µl/min for mice and 100 µl/min for rats.

As mentioned above, micturition in humans and rodents differ significantly. For example, ATP is a major contributor to the detrusor contraction in mice and rats, whereas in humans, the bladder contraction, under physiological conditions is mainly mediated by acetylcholine[4]. However, the possibilities to perform invasive measurements that are generally prohibitive in the clinical context and the use of genetically modified animals, allow exploring the current boundaries of bladder pathophysiology and pharmacology. In this respect, significant advances have been recently obtained in elucidating the role of muscarinic[14], prostaglandin[15] and adrenergic receptors[16], neuropeptides[17], Ca^{2+}-activated K^+ channels[18] and TRP channels[3,5,6].

Disclosures

No conflicts of interest declared.

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