Bladder Physiology and Application

W12, 15 October 2012 14:00 - 15:30

Start	End	Topic	Speakers
14:00	14:05	Introduction	• Longkun Li
14:05	14:30	Neurologic physiology in urinary bladder	• Sivert Lindstrom
14:30	15:00	Regulation in cystogenic excitation	• Longkun Li
15:00	15:10	Questions	All
15:10	15:30	Neuromodulation	• Chonghe Jiang

Aims of course/workshop

Because the urinary bladder can contract voluntarily through myogenic excitation, it would be reasonable to predict that the bladder has the potential for self-excitation. The physiological effects of the hyperpolarization-activated nucleotide-gated (HCN) channel in initiating and modulating bladder excitability need to be investigated. Moreover, the interstitial cells of Cajal (ICCs) in the bladder have been suggested to participate in the bladder pacemaking and signal transmission. However, the relationship between the ICCs and HCN channels needs to be clarified. In this study, the HCN channel and its subtypes in the bladder, as well as their relationships to the bladder ICCs, were investigated.

Educational Objectives

So far, few studies have focused on the HCN channels located in the urinary bladder, especially their biophysical properties and their role in cell excitation. Bladder smooth muscle cells (BSMCs) comprise the basic contractile unit in the urinary bladder, with tight connections to nerve fibres and kit-positive ICCs. It can be supposed that bladder sensation, afferents, efferents and contraction represent an integrated network that might be regulated by a functional tri-unit consisting of nerves, ICCs and BSMCs. Whether there are any changes in the HCN channels in the pathological conditions and how this channel function should be a potential field in understanding the basic physiological properties of bladder in filling and voiding phase.

Basic research studies on the bladder excitation regulation

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Bladder excitation can be regulated by neurogenic/myogenic factors. Are there any relationships between the neurogenic and myogenic mechanisms. Are there any other mechanism for bladder excitation regulation. In the past decade, many centers, including our laboratory, have clarified some further mechanisms.

1. ICC may behave as the mediator of the bladder excitation.

①Interstitial cells of Cajal, which act as pacemaker in gastrointestinal tract, have also been found in the bladder tissue. We successfully identified bladder ICC in mixed primary culture.

②Most of ICCs locate in sub-mucous membrane, along the longitude of smooth muscle bundles, between smooth muscle bundles or along the blood vessels, and the cells in different locations may play a different role;

③There are two existent fashions of ICCs in rat bladder: single or conjuncted closely each other. In normal bladder, most of the ICCls are single, and in unstable bladder, there are more ICCs with network formation, hinting their potential functions.

④There is a strong positive correlation between the density of ICCs and detrusor excitability, and the strips contraction and myoelectricity potential can be obviously affected by Glivec, which indicated that there may be close relationship between ICCs and the function of detrusor.

⑤Hyperpolarization-activated cyclic nucleotide gate(HCN) ion channel, which was specified in generating pacemaking current, was found on the membrane of ICC: we used double-labeled IF by c-Kit and HCN antigen to detect it, so the capcity of ICC to generate pacemaking current was found.

⑥We successfully create the rat model with detrusor hyperreflexia and detrusor arereflexia, and have found that there are significantly more ICCs in bladder of SSCI rats than those in the normal and post-cystostomy rats bladder. The quantity of ICCs in normal bladder is similar to that in post-cystostomy bladder. There are significantly decrease of ICCs number in SCI rats bladder.

⑦We successfully freshly dispersed and identified ICCs in rats bladder. The spontaneous excitability of ICCs in SSCI rats is higher than that in normal and post-cystostomy rats. The spontaneous excitability of ICCs in normal rats is similar to that in post-cystostomy rats. The spongtanous excitability of ICCs in SCI rats is lower than that in normal and post-cystostomy rats. The spontaneous calcium waves of ICCs in bladder is suppressed by Glivec with dose dependent. Glivec in low concentration(1×10^{-6}mol/L) suppress the spontaneous calcium waves of ICCs in bladder with detrusor hyperreflexia, while 5×10^{-5}mol/L Glivec suppress the spontaneous calcium waves of ICCs in bladder with detrusor areflexia.

Each group has different spontaneous contractions of bladder in rats with unvarying preload (1g). The amplitude of spontaneous contractions of bladder with detrusor hyperreflexia in rats is higher than that in bladder with detrusor areflexia.

⑧The amplitude of spontaneous contraction of detrusor strips is inhibited by Glivec application with dose dependent. Glivec in low concentration(1×10^{-5}mol/L) suppress the amplitude of spontaneous contraction of detrusor strips with detrusor hyperreflexia in rats, while 5×10^{-4}mol/L Glivec suppress the amplitude of spontaneous contraction of detrusor strips with detrusor areflexia.

Glivec (10mg/kg) increased the bladder capacity and compliance in rats with detrusor hyperreflexia and did not influence the bladder capacity and compliance in rats with detrusor areflexia. The spontaneous contraction of bladder can be suppressed by Glivec (20mg/kg) during the storage phase.

2. cell-cell communication between the ICC/ICC, ICC/BSMC (bladder smooth muscle cell), and BSMC/BSMC

①We established the effective method to investigate the morphology of ICC in the bladder: ICCs were confirmed to exist in suburothlium layer and the border of detrusor smooth muscle in bladder as a network through KIT immunohistochemistry.

②There was tight connection between ICC and detrusor smooth muscle cell: ICCs were close to smooth muscle cells and they were intercrossed to each other when double-labeled IF was applied for analysis. Moreover, gap junction was detected by transmission electron microscope (TEM).

③Signal transmission was found from ICC to detrusor smooth muscle cell: We found the cell-cell signal could be successfully transformed from ICC to BSMC using fluorescence recovery after photobleaching (FRAP), which might be the functional basis for ICC to be pacemaker cell to initiate the action potential of detrusor smooth muscle cells.

④A mode for ICC's functional inhibition was established: The effects of blocking agent Glivec were mainly on the calcium influx of ICC which provided us a new mode to investigate the function of smooth muscle cells with ICC's function decreased.

Changes of detrusor function were detected on the level of cell, tissue, and in vivo bladder. Calcium influx and signal transmission from ICC to detrusor were inhibited significantly and contractile amplitude of detrusor was also decreased significantly in muscle strip and bladder as a whole. That indicated that ICC would

play a crucial important role in the movements of bladder.

3. neural innervation and ICC.

①The ICC-like cells do locate in rat ladder, most of the cells locate in sub-mucous membrane, along the longitude of smooth muscle bundles, between smooth muscle bundles or along the blood vessels, and the cells in different locations may play a different role.

The density of nerve fibre decreases significantly in unstable bladder and there is a strong negative correlation between the ratio of nerve fibre to ICC and detrusor excitability.

②We found that M2 and M3 but not M1, M4 and M5 receptor subtypes were expressed in bladder ICCs of SD rat by double lable of immunofluorescence.

In vitro application of Carbachol, an agonist of M receptor, significantly enhanced [Ca2+]i in cultured ICC.

The Carbachol-induced excitability in cultured ICCs was mildly inhibited by Methoctramine, the antagonist of M2 receptor, while it was significantly inhibited by 4-DAMP, the antagonist of M3 receptor.

③The bladder strips showed spontaneous contractions under a fixed tension (0.75g). Gradually increasing the concentration of Carbachol could induce significant increasing in the amplitude of whole contractions and spontaneous contractions. Moreover, the frequency of spontaneous contractions showed high frequency in first and then down later.

④The amplitude/frequency of spontaneous contraction and the tension of the strips induced by Carbachol could be significantly inhibited by 4-DAMP but mildly inhibited by 4-Methoctramine.

Cholinergic nerve stimulate can excite ICCs in bladder mainly via M3 muscarinic receptors.

ICCs in bladder might play roles in the regulation of detrusor contraction by the interaction with cholinergic nerve.

4. in vivo and in vitro regulation of stretch load on the bladder ICC.

①Long-term obstruction following PBOO caused morphological changes and increasing quantity of ICC, which may play a role in the pathogenesis of DO. Freshly dispersed ICC from DO bladders showed spontaneous calcium waves with high frequency and lower amplitude comparing to those from DS and control bladder, which indicated that ICC have different excitability in DO bladders.

②Static mechanical stretch device for in vitro cultured cells was successfully constructed. Mechanical stretch could be applied approximate 10-30% step-wisely and extension was subjected uniformly to the silicone membrane, and the cells cultured on it. Hypotonic distension could also expand the cell volume to obtain

mechanical stretch in vitro. However, the non-uniform swelling and extracellular environment changing underline hypotonic restrict its application.

③Freshly isolated ICC from DO bladders showed spontaneous calcium waves with higher frequency and lower amplitude comparing to those from stable and control bladder.

④Stretch-induced [Ca2+]i transient could be detected in cultured bladder ICC when 20-30% extension was applied to the cultured cells via lengthening the silicone membrane. The similar calcium augment were also detected in the cultured smooth muscle cells with longer responding time comparing to ICC. Stretch-induced calcium transient(SICT)generated in ICC could transfer to adjacent smooth muscle cells through cell membrane connection.

⑤ The amplitude of SICT was significantly reduced when removing the extracellular Ca2+ or exposed to ruthenium red . 2-APB nearly abolished the augment of [Ca2+]i response to mechanical stretch application.

⑥Cultured bladder ICC showed mechanical sensitivity and presented enhanced [Ca2+]i augment under stretch load. ICC were more sensitive than SMC to mechanical stretch and the calicium waves could transfer from ICC to adjacent SMC. Mechano-sensitive role of ICC provide a novel mechanism underling myogenic contraction.

IP3 sensitive Ca2+ stores release compose the major part of initiation of [Ca2+]I augment in responding to mechanical stretch.

5. self-excitability of the BSMC.

①There is concordance for detrusor myogenic change after suprasacral cord injury and BOO,just as DI,the occurrence of DH is related to the detrusor myogenic chang after suprasacral cord injury .

②The Detrusor compliance decrese after suprasacral cord injury or BOO. The detrusor occur following change,and the excitability increase after suprasacral cord injury or BOO.

The character change of T-type calcium channel leads to the increase of detrusor excitability probably, and the change of L- type calcium channel has little effect in the development of DH or DI.

③The express of detrusor connexin43 increase after suprasacral cord injury or BOO.This change indicates the increase of excitation conduction intercellular,and the development of DH or DI is related to the increase of excitation conduction too.

④The increase of ß-actin, type-□ and type-□collagen fibrils lead to the decrease of bladder compliance.

There is common pathogenesis in the development of DI and DH,that is detrusor myogenic change is related to DH or DI closely.

Bladder cooling reflex and its clinical application

Bladder cooling test (BCT test or ice water test) is a very useful test in diagnose the neurogenic problems in patient with bladder dysfunction. It was first introduced by Dr. Bors and Blinn in 1950s. However, it was not applied widely until 30 years later, an extensive studies have been done by professor Fall, Lindstrom and their colleagues.

In my presentation, I will make a simple report about our work in bladder cooling reflex, including 1/ experiment studies (cats & human); 2/ suggested procedure; 3/ clinical outcome (adults, children).

First, what is the bladder cooling reflex and what is the bladder cooling test. I will show you a recording for a 6 years old patient with myelomeningocele. As you can see, there is no detrusor contraction with warm bladder infusion but big contraction induced by the same volume of cold infusion. It is defined as positive bladder cooling test.

To identify the bladder cooling reflex pathway we made a series experimental study both in animal and human. The experiment setup in the cat will be presented. We inserted the catheters into the bladder and urethra. Another bladder catheter was for pressure recordings. The bladder pelvic nerve was dissected and some small filament were cut for recording afferent and efferent activities. Hypogastric nerve to the bladder was cut to remove its influence to the bladder.

In the experiment, we were trying to solve following problems. 1) What is the triggering stimulus for the bladder cooling reflex? Are they bladder tension or bladder cooling; 2) Where are the cooling receptors? Are they bladder and urethra walls or structures around the bladder; And 3) What type of receptors are involved? Are they Aδ or C fiber, or nociceptors (pain receptors).

The recordings of bladder and urethra cooling reflex in the cat will be shown. With the small volume (5 ml) injection, only the cold induces the bladder contraction and efferent responses (also for the urethra cold perfusion). 5 ml is not enough to activate normal micturition bladder contraction but cold can do that. It means that the bladder and urethra have additional cold receptors other than normal micturition mechanoreceptors.

When we increased the injection volume to 15 ml, the bladder starts to contract even with warm saline. However, there were two typical differences between the cold and warm stimulus. Warm infusion induced less bladder contraction but more afferent activity compare with the cold infusion which means that less sensitivity in bladder cooling; another character is that a long last effect was showing by the cold infusion. All these findings indicate that bladder cooling receptors are different from micturition mechanoreceptors.

The second question is where the cooling receptors are located? As you know, other organs around the bladder could be cooled also by the bladder cooling. To make sure if the cold receptors is in the bladder, we used local anaesthesia – Xylocaine to black the bladder nerve to see what happen? You can see that bladder cooling reflex had been suppressed compare with the control. And again, cold flow to the urethra still evoked cooling reflex, because the

urethra was not anaesthetized. Now we can say that the bladder cooling reflex originates from sensory receptors in the bladder and urethral walls, not somewhere else.

Another question is what type of receptor involved? We know that the normal micturition reflex is triggered by the activation of bladder mechanoreceptor Aδ afferent. The reflex pathway is travel via supra spinal pontine micturition center, while bladder cooling reflex was proposed via spinal C fiber pathway under the control of supra spinal micturition center. The positive bladder cooling test is due to the disturbances in the spinal upper motor pathway and lost the descending control of the bladder cooling reflex.

From the slide we can see that the bladder pelvic nerve responses by the stimulation of same side and contralateral side of nerve branch. There are Aδ and C reflex discharges. After cutting the spina cord at the level of T10, the Aδ discharges was disappear but C reflex was remain, indicating the bladder cooling reflex is spinal reflex pathway.

Another slide shows that after spinalization, the C fiber discharge was increased by cold flow to the urethra but not by warm solution. It was also facilitated by menthol flow. Menthol is well known a substance to increase the sensitivity of cooling receptor in the skin, it is also work in the bladder.

In the following experiment, we were trying to detect the temperature threshold for the bladder cooling reflex in the cat by injection with different temperature. You can see that the lower temperature was the better cooling response. The temperatures were measured immediate after the infusion withdraw. As you know, the actual stimulation temperature in bladder wall was higher than the infusate because it was warmed by the catheter, surround tissue and residual volume in the bladder. Therefore, we measured the fluid for both in and out temperature and took the average value for the real stimulation temperature. Fig B shows after correction, the bladder stimulation temperature induced the cooling response. The highest temperature that induces cooling response (T threshold) was 32 ^{o}C. Obviously, this cooling was not painful and nothing to do with nociceptive stimulation (the cold pain stimulation is under 20^{o}C).

Why the BCT test is positive in the cat during the anaesthesia. For this question, we made a separate experiment in adult cat to show that the bladder cooling reflex is suppressed in awake situation and reappeared when the animal was falling a sleep or during narcotic sleep – anaesthetized.

In summary, bladder cooling reflex is activated by cold receptors, while micturition reflex by tension receptors; bladder cooling reflex is menthol sensitive while micturition reflex not; bladder cooling reflex is conducted by C afferent, while micturition reflex by Aδ, both of them in the same pelvic nerve but different central nerve system: spinal reflex pathway for cooling reflex, and supra spinal reflex pathway for normal micturition reflex. In children, there is evidence that before development of voluntary micturition control, the test is positive and coverted into negative after that in about 2 years old. That is the age dependences.

The following recordings show a girl with a positive BCT at the age of 4 moths became negative after 2 years when she got voluntary control of the micturition. Supression of the BCT at about age 2 years represents normal physiological development

Statistically, we shows the results in a group of children (60) with various nonneurogenic lower urinary tract problems. You can see that the most positive were under the age of 2. Different from the adult patients, some positive cases after 2 years old are not the sign of neurogenic problems. It was confirmed later as the sign of good prognosis for urge incontinence problems. The figure on the right shows outcome of repeat BCTs in relation to age in children with idiopathic incontinence. They were all converted from positive to negative after the age of 2.

In the clinical research work, we checked the temperature threshold of cooling test in patients. The figure on the left was a typical recording of positive BCT in a patient with upper motor neuron lesion by different temperature for bladder infusions. Figure in the right was the recordings from 16 patients with overactive bladder. One can see that the lower temperature was the better cooling response. But the majority was activated with the infusion temperature at 10^oC. Compare with the cat, the human bladder cooling reflex has similar temperature threshold. Ice water is not necessary for BCT, $4 – 8^o$ C will be enough.

Our another study is to show how much infusion volume needed for the BCT. As you can see in the scatter plot, the larger bladder capacity is, the more cold infusate is needed. Nervetheless, 30 to 40% of capacity volume is reasonable for cold infusion. If cystometric bladder capacity is < 200 ml, 100 ml saline or 50 % of capacity volume is needed.

How about the infusion speed for BCT? Actually, it is not very important compare with the infusion temperature. One can see that in the figure, there are not much differences from 100 to 300 ml per minutes. But if the infusion speed is too low (< 50ml/min), the infusate temperature become too high (above 23^oC) to activate cooling reflex.

Except the cold provoked urine leakage around the catheter, which is always considered as positive. We also provide the criteria for the detrusor pressure in BCT test. Both histogram and scatter plot show that detrusor pressure $\geqslant$ 30 cm H_2O are classified as BCT positive.

In summary of suggested procedure for BCT: Infusion volume is100 ml saline or 50 % if cystometric bladder capacity < 200 ml; Temperature is 4 to 8^o C, directly from refrigerator; Infusion speed is 200 – 300 ml/min; Criteria for BCT positive is leakage or detrusor pressure $\geqslant$ 30 cm H2O. There are some exceptions for children, which include the pre-test and fluid out temperature should be less than 22^oC, otherwise, repeat.

How about the clinical value of BCT? We collected some of main publications about the outcome of BCT including a total 2019 cases with variable diagnoses.

The patients with neurogenic normal patients all with BCT negative. In the spinal injury group, there is clear opposite result between upper and lower motor lesions. Upper motor neuron lesions were positive and lowers were negative. Since no bladder contraction in the patients with lower motor neuron lesion, cold can not induce detrusor contraction either. BCT positive also dominant in other central nerve system disease like stroke, Parkinson´s disease and multiple sclerosis, the negative part may be due to the technical error or the degree of disorders.

In patients with voiding dysfunction, all the stress incontinence cases are negative since there are nothing related with nerve system. Positive are dominant in all these overactive bladder

explain that the abnormal voiding is mainly triggered by bladder C reflex spinal pathways or they lost the cortex control. The same reason for the other disease with positive test.

In conclusions:

- The BCT is a simple and rapid add-on test
- Its outcome is easy to interpret
- It is well tolerated by patients
- The underlying physiology is reasonably well known
- It can in doubtful cases differentiate upper from lower motor neuron lesions
- It can varify a neurogenic etiology in certain types of urge incontinence

Notes

Record your notes from the workshop here