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ABSTRACT

Aims: To discuss (1) animal models for investigating bladder afferent pathways from the spinal cord to the brain and (2) animal models of stress urinary incontinence (SUI) with a special emphasis on functional and histopathological characteristics of each model. **Methods:** Literature review of spinal mechanisms of bladder afferent pathways and animal models of SUI. **Results:** Electrophysiological studies in the rat using pelvic nerve stimulation and recording of evoked potentials in the periaqueductal gray (PAG) prove to be a valuable tool to examine spinal mechanisms of bladder afferent pathways. Animal models of SUI in the rat include vaginal distention as simulated birth trauma, pudendal nerve crush or transection, urethral sphincter injury by electrocauterization, transabdominal urethrolysis, periurethral botulinum-A toxin injection, and pubo-urethral ligament transection. Functional and histopathological changes in the continence mechanism after injury are different between models. **Conclusions:** Using animal models for sensory neurons, intrathecal and intravenous administration of certain drugs can be tested whether they affect the bladder afferent pathways from the spinal cord to the PAG. Animal models of SUI can serve as a tool to develop new pharmacologic therapies or periurethral injection therapies using stem cell implants.

INTRODUCTION

Bladder afferent pathways are very important as a target of the treatment of overactive bladder. Bladder afferent pathways start from the urothelium, and sensory nerves (primary bladder afferents) reach the dorsal root ganglia and then bladder afferent pathways travel through the spinal cord and reach the pons, midbrain and higher brain (Fig.1). Because primary bladder afferents are described in another chapter ("Afferent discharge" by Stefan De Wachter), we focus on higher neural mechanism of bladder afferent pathways from the spinal cord to the brain, especially to the midbrain.

Stress urinary incontinence (SUI) is a highly prevalent disorder in female population and has a significant impact on the quality of life in affected women. Although effective surgical modalities are available for SUI, animal models for SUI have been pursued to detail the pathophysiology of SUI and to explore the possibility of development of pharmacologic therapy and stem cell implants.

ANIMAL MODELS FOR SENSORY NEURONS

Afferent nerves from the bladder enter the dorsal horn and project to regions of the spinal cord that contain interneurons located at dorsal commissure (DCM) and sacral parasympathetic nucleus (SPN).[1] Dorsal horn, DCM and SPN all contain interneurons with ascending projections. And these neurons are the site of origin of ascending pathways that project to various structures in the brainstem or midbrain. Recent neuroanatomical studies and functional brain imaging in human show that the periaqueductal gray (PAG) is a crucial center for bladder afferent pathways[2-7] However, there are only limited data about the intraspinal neural mechanism and receptors that are involved in spinal ascending pathways.[8,9]

Fig.2 shows an experimental schema to examine spinal ascending pathways from the pelvic nerve (PLN) to the PAG in the rat. Under anesthesia, a tracheostomy tube was inserted to facilitate respiration and permit artificial ventilation after neuromuscular blockade. Through a midline abdominal incision, the PLN was isolated and prepared for electrical

stimulation. The urethral catheter was connected to a pressure transducer to monitor intravesical pressure. Then the rat was placed in a stereotaxic apparatus and a small craniotomy was performed to insert a recording electrode into the PAG. After completion of the surgical procedures, the animal's lower body was rotated 120° to expose the bladder and PLN. The PLN was transected and its proximal end was placed on a bipolar silver electrode for stimulation. A fine monopolar recording electrode was inserted stereotaxically into the medial part of the dorsal pontine tegmentum in 0.25- or 0.5-mm steps. Sites in the pons and PAG were identified where PLN afferent stimulation (1-15 V, 0.05 ms pulse duration at 100-300 Hz, 5-30 ms train duration) evoked field potentials. Intrathecal catheter was used to evaluate effects of intrathecal injection of various drugs (Fig.2). These experimental setups are very valuable to examine spinal mechanisms of bladder ascending pathways.

Recording examples are shown in Fig.3 and Fig.4. PLN stimulation evoked short latency (10-22 ms) negative field potentials (85 ±4 μV) in a relatively limited area of the PAG; 8.4~9.0 mm posterior from the Bregma, 0.5~1.5 mm lateral from the midline, and 4.2~6.0 mm in depth from the brain surface.[8,9] It is well known that glutamatergic mechanisms play an important role in the reflex pathways controlling the lower urinary tract.[1] A previous study evaluated the contribution of spinal glutamate receptors (AMPA and NMDA receptors) to the ascending limb of the micturition reflex pathway in the rat.[8] Intrathecal injection of AMPA glutamate receptor antagonist (LY215490, Fig.3) as well as NMDA receptor antagonist (MK-801) reduced the amplitude of the evoked potentials in a dose-dependent manner without affecting the latency of the evoked potentials. Therefore it is suggested that both AMPA and NMDA glutamatergic transmission plays a key role in the spinal processing of afferent input from the bladder. Another study examined the role of spinal 5-HT_{1A} receptors in the control of micturition reflex pathway in the rat.[9] Intrathecal injection of a selective 5-HT_{1A} receptor antagonist (WAY100635) at the doses 30 μg and 100 μg did not change the amplitude of the evoked potentials in the PAG (Fig.4), suggesting that spinal 5-HT_{1A} receptors are not involved in the bladder afferent pathway. Using this model, intrathecal as well as intravenous administration of certain drugs can be tested whether they affect the bladder afferent pathways from the spinal cord to the PAG.

ANIMAL MODELS OF SUI

SUI is a significant medical problem affecting a large population of women. SUI is usually caused by one or more of the following factors: childbirth, aging, and hormone deficiency, which in turn result in altered anatomy and function of the urethra, vagina, and supporting structures of the pelvic floor.[10] Other factors known to contribute to the development of SUI are obesity, chronic cough, multiparity, smoking, and constipation. However, because of the difficulty in obtaining tissue from human females, studies of the effects of age and parity and other factors on the continence mechanism is lacking.[11] Lin et al first created a rodent model that permits an examination of the effects of artificially induced vaginal trauma on adjacent structures and the continence mechanism.[10] Four weeks after simulated birth trauma, SUI was noted in 19 of 48 experimental rats (40%).[10] Following the study, several investigators have used this rodent model of SUI that is created by vaginal distention for simulated birth trauma.[11-15] Other investigator introduced other rat models of SUI by pudendal nerve crush[16] or transection,[17] urethral sphincter injury by electrocauterization,[18] transabdominal urethrolysis,[19] periurethral injection of botulinum-A toxin,[20] transecting the pubo-urethral ligament,[21] or urethrolysis followed by injection of myotoxin cardiotoxin.[22] All these models except vaginal distention do not simulate an injury that occurs naturally in childbirth. Advantages and disadvantages of various rat models of SUI are well summarized in a previous review article.[23] The development of a reproducible animal model of SUI would lead to better understanding of pathophysiology of SUI and to explore new treatment strategies for SUI.

Methods to create SUI model in the rat

Vaginal distention

Under anesthesia, a 10~12Fr balloon catheter with tip cut off was inserted into the vagina. The Foley balloon was inflated with 2~4 ml water. After 2~4 hr of vaginal distention, the balloon was deflated and removed. SUI was evaluated 4 days,[12-15] 4 weeks,[10] or 8 weeks[11] after vaginal distention depending on the experimental protocol. Cannon et al analyzed the effect of vaginal distention with different duration; brief (0.5 hr),

intermittent (cycling inflated /deflated for 0.5 hr), and prolonged (1 hr) distension.[12] Abdominal leak point pressure (ALPP) in the prolonged vaginal distention group (31.4±1.7 cmH_2O) was significantly lower than in the sham control group (41.1±3.2 cmH_2O), while there were no significant differences in ALPP between the brief (36.9±4.2 cmH_2O) or intermittent (41.2±2.3 cmH_2O) vaginal distention and the sham control groups.[12] Thus vaginal distention for at least 1 hr **was** required to create SUI model. Sievert et al modified the technique of vaginal distention to simulate difficult labor in human.[11] They used primiparous pregnant rats, and immediately after delivery animals were separated into 2 groups with half in each group undergoing intravaginal balloon inflation. Four weeks after parturition every second animal in each group underwent ovariectomy (simulated hormone deficiency), resulting in 4 groups; group1- delivery, group 2- delivery plus vaginal distention, group 3- delivery plus ovariectomy and group 4- delivery plus vaginal distention plus ovariectomy. SUI induced by sneeze stress test was noted in 16.7%, 58.3%, 16.7% and 72.7% of group 1 to 4, respectively. Thus vaginal distention model (group 2 and group 4) resulted in s significantly higher incontinence rate than delivery alone (group 1) and delivery plus ovariectomy (group 3), while delivery plus ovariectomy (group 3) did not result in a higher incontinence rate than delivery alone (group 1).

Pan et al compared the effect of different duration (1 vs 4 hours) of vaginal distention on ALPP.[24] They found that ALPP was significantly decreased in both distention groups 4 days after vaginal distention, but 10 days after vaginal distention ALPP was significantly decreased in the 4-hour but not in the 1-hour distention group. Six weeks after vaginal distention, ALPP in both distention groups was not significantly different from sham control group. These findings suggest that vaginal distention is not a durable SUI model and recovery time of ALPP after vaginal distention is different depending on the duration of vaginal distention. This should be reminded when making experimental protocols of SUI model using vaginal distention.

Lin et at carried out histopathological examination of the urethra and pelvic floor tissues 4 weeks after vaginal distention.[10] They found a significant decline in urethral wall musculature (both smooth and striated) in incontinent rats. The number of ganglion cells in the neural plexus posterolateral to the vagina was significantly less in the vaginal distention rats than the control rats. In the levator ani, muscle necrosis and

degeneration, irregular shape and size of muscle fibers were noted. There also was a change in the ratio of the type I (slow-twitch) and type II (fast-twitch) muscle in the levator ani with a significant increase in the ratio of type I muscle in incontinent rats; 0.05% type I and 99.95% type II in control rats, 22.79% type I and 77.21% type II in incontinent rats.

Sievert et al performed ultrastructural and immunohistochemical studies on urethral and bladder smooth muscle in rats that had undergone vaginal distention and revealed various degrees of changes in plasma membrane caveolae, caveolin-1 and 3, neuronal nitric oxide synthase, and urethral and bladder smooth muscle.[11] Other studies also showed that vaginal distention caused myogenic, neurogenic and ischemic changes of the urethra, bladder, vagina and levator muscles.[23,24] Yoshimura and his associates examined immunostaining of PGP 9.5 (neuronal marker) of the urethra taken from the sham control and vaginal distension rats. Compared with the sham control rat, PGP staining in the urethral wall was significantly reduced in the vaginal distention rat, suggesting the denervation of the urethra after vaginal distention (personal communication).

Taken together, vaginal distention model that simulates birth trauma, can cause pathologic and functional changes in the urethra musculature, levator muscles, pelvic ganglia, neurotransmitter, and transmembrane signaling mechanisms that contribute to the development of SUI.

Pudendal nerve crush or transection

Damaser et al developed an animal model of SUI using pudendal nerve crush.[16] The pudendal nerve was located in the ischiorectal fossa and the whole pudendal nerve was crushed twice for 30 seconds bilaterally just proximal to the branch point of the obturator nerve. ALPP was significantly decreased 4 days after pudendal nerve crush (29.3±3.4 cmH_2O) compared with sham operated controls (44.3±3.4 cmH_2O). Two weeks after pudendal nerve crush ALPP returns almost to normal values.[23] Rats with pudendal nerve crush had significantly fewer nerve fascicles near the external urethral sphincter (EUS) than controls (about 34% reduction) and had about 13% of degeneration of nerve fascicles.[16]

Peng et al reported the value of pudendal nerve transection as a durable SUI model in the rat.[17] Six weeks after pudendal nerve transection ALPP was significantly decreased in the unilateral transection group (31.7±1.4

cmH_2O) and further decreased in the bilateral transection group (25.6±2.6 cmH_2O) compared with sham operated control group (41.2±1.9 cmH_2O). In addition, pudendal nerve transection also caused electromyographic abnormalities of the EUS and striated muscle atrophy in the EUS.

Electrocauterization

Chermansky et al reported the use of electrocauterization of the urethra as a durable SUI model in the rat.[18] This electrocauterization model produced low leak point pressure (about 50% of sham operated controls) that were maintained for up to 16 weeks. Histological findings showed muscle disruption of the EUS and nerve damage (denervation) within the mid-urethra 16 weeks after electrocauterization.

Urethrolysis

Rodriguez et al developed a durable SUI model in the rat by using transabdominal urethrolysis.[19] Following a lower abdominal incision, the proximal and distal urethra was detached circumferentially by incising the endopelvic fascia and detaching the urethra from the anterior vaginal wall and pubic bone by sharp dissection. Compared with baseline values, ALPP (19.4±3.8 vs 9.8±3.2 cmH_2O) and retrograde urethral perfusion pressure (22.6±5.0 vs 11.2±4.9 cmH_2O) was decreased significantly by 1 week after urethrolysis and these changes were maintained for up to 24 weeks. Changes seen in urethral resistance appeared to be mediated by apoptosis, decreased neuronal mass, and smooth muscle atrophy in the urethra.

Kinebuchi et al reported another durable SUI model in the rat with more damage to the urethra.[22] They combined urethrolysis with an injection of a myotoxin cardiotoxin (CTX) into the distal urethra under the pubic bone. CTX is widely used to induce experimental damage of skeletal and cardiac muscle. Its action is reversible, and therefore they performed urethrolysis in conjunction with the injection of CTX to produce damage directly to the striated muscle of the urethra. One week after the urethral injury, leak point pressure was decreased about 50% and that was maintained for up to 12 weeks.

Periurethral injection of botulinum-A toxin

Takahashi et al injected 10 U/200 μl botulinum-A toxin periurethrally at

the mid-urethra to create a durable SUI model in the rat.[20] Two weeks after injection, leak point pressure was significantly decreased compared with sham controls (58.7±6.2 vs 120.7±13.0 cmH_2O), and cross-sectional area of smooth muscle layer and striated sphincter was decreased to 57% and 67% of sham controls, respectively. Six weeks after injection, mean leak point pressure recovered to the level of 86.0 cmH_2O. Since periurethral botulinum-A toxin injection does not require an abdominal incision, it is an easy and feasible model for inducing the impaired urethral sphincter.

Pubo-urethral ligament transection

Kefer and Daneshgari introduced another rat model of SUI by transecting the pubo-urethral ligament (PUL).[21] PUL-transected rats demonstrated significantly decreased leak point pressure compared to the sham control rats at 4 days (16.3 vs. 36.6 cmH_2O) and 10 days (17.6 vs. 31.2 cmH_2O) after the procedure. PUL transection might simulate urethral hypermobility in women with SUI.

Methods to induce stress condition and record leak point pressure in the rat

In many studies, rat whisker was cut and inserted into the nostril to induce the sneeze reflex (Fig.5) with the rat under urethane anesthesia. The urethral meatus is observed for urinary leakage as the rat sneezes.

Fig.6 shows measurement of sneeze-induced leak point pressure. Sneeze reflex is induced many times. The lowest intravesical pressure inducing leakage is measured as sneeze-induced leak point pressure. During measurement, bladder volume is maintained around 0.2 to 0.4 ml with Evans blue or methylene blue for easy recognition of fluid leakage.

CONCLUSIONS

Spinal ascending pathways are critical components of bladder afferent pathways, and further researches are warranted to examine receptors and neural mechanisms involved in spinal ascending pathways. Electrophysiological methods described here are important to analyze the functional aspect of spinal ascending pathways.

Animal models of SUI are suitable to examine structural and functional changes in pelvic organs that contribute to the development of SUI. The rat model of simulated birth trauma by vaginal distension shows evidence of pressure-induced ischemia, pelvic floor injury and dysfunction of the urethral continence mechanism. With further understanding of the pathophysiology of animal models of SUI, it may be possible to prevent these injuries in the acute phase and to prevent late consequences (development of SUI later in life) in humans. Animal models of SUI can serve as a tool to develop new pharmacologic therapies or periurethral injection therapies using muscle stem cells.

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AUTHOR DISCLOSURES

Hidehiro Kakizaki–Consultant: Astellas Pharma Inc., Pfizer Japan Inc., Kissei Pharmaceutical; Speaker honorarium: Astellas Pharma Inc., Pfizer Japan Inc., Kissei Pharmaceutical, Taiho pharmaceutical, Ono Pharma. Masafumi Kita–None. Naoki Wada –None.

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FIGURE LEGENDS

Figure 1 Neural pathways connecting lower urinary tract and midbrain
EUS: external urethral sphincter

Figure 2 Scheme to examine spinal ascending pathways. Adapted from Noto et al (Brain Res 549: 95-105, 1991, Fig.1)

Figure 3 Effect of intrathecal (i.t.) administration of AMPA glutamate receptor antagonist (LY 215490) on PLN-evoked potentials in the PAG (upward trace indicates negative potentials). Each record is made by averaging 10 successive evoked responses. (From Kakizaki et al,[8] J Pharmacol Exp Ther, 1998, used with permission)

Figure 4 Effect of intrathecal (i.t.) administration of a selective 5-HT_{1A} receptor antagonist (WAY 100635, 30 and 100 μg) on PLN-evoked potentials in the PAG. (From Kakizaki et al,[9] Am J Physiol Regul Integr Comp Physiol, 2001, Am Physiol Soc, used with permission)

Figure 5 Rat whisker is cut and inserted into the nostril to induce the sneeze reflex.

Figure 6 Scheme to measure sneeze-induced leak point pressure
Pves: vesical pressure

Figure 1

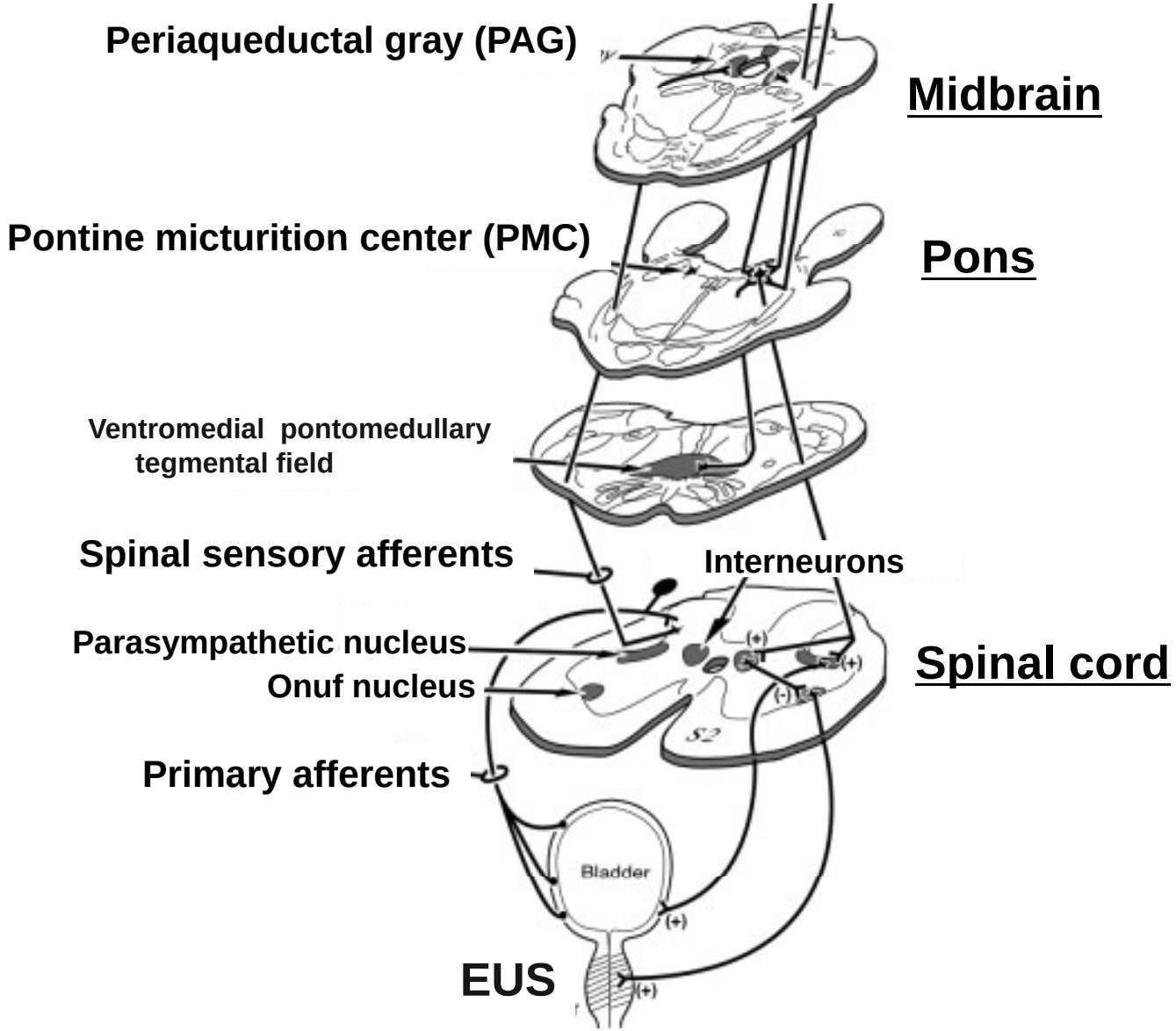

Periaqueductal gray (PAG)
Midbrain
Pontine micturition center (PMC)
Pons
Ventromedial pontomedullary tegmental field
Spinal sensory afferents
Interneurons
Parasympathetic nucleus
Onuf nucleus
Spinal cord
Primary afferents
Bladder
EUS
S2
(+)
(-)

Figure 2

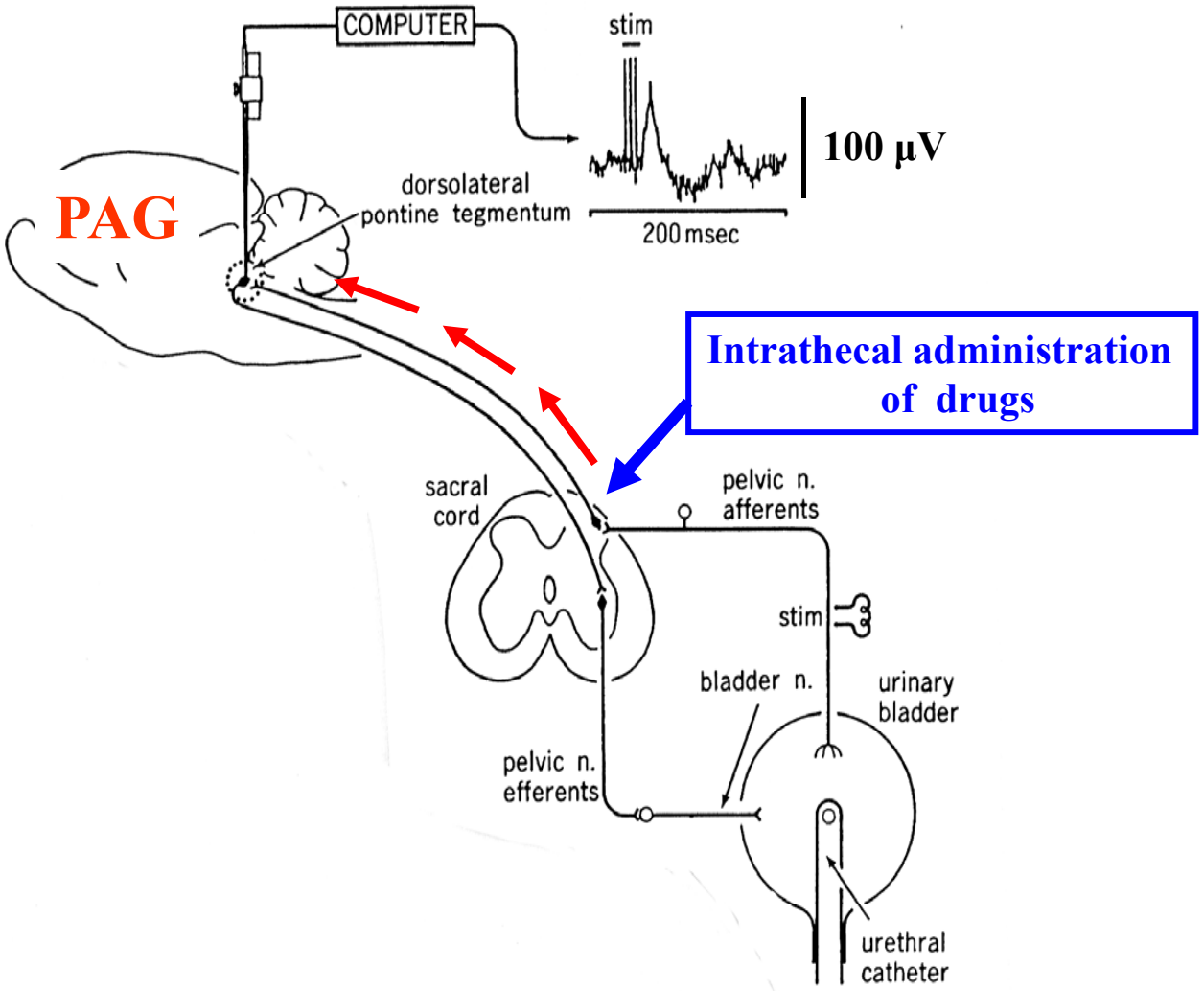
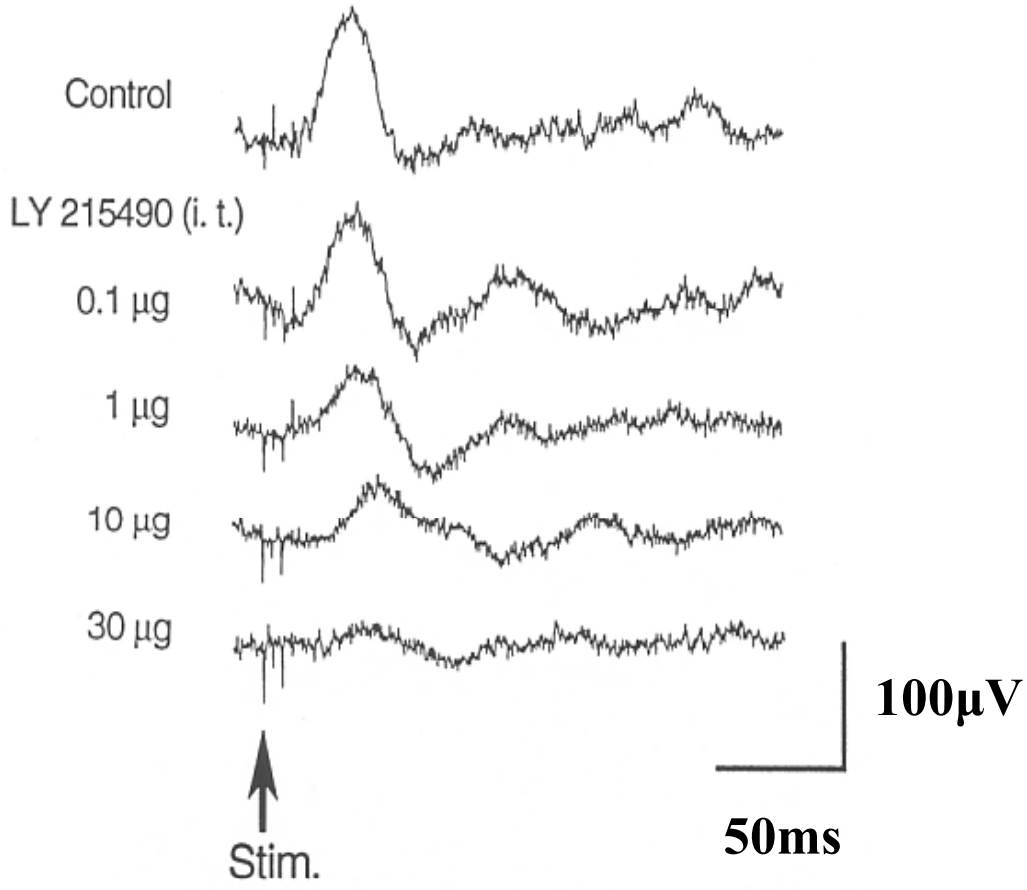

COMPUTER
stim
100 µV
200 msec
dorsolateral
pontine tegmentum
PAG
Intrathecal administration
of drugs
sacral
cord
pelvic n.
afferents
stim
bladder n.
urinary
bladder
pelvic n.
efferents
urethral
catheter

Figure 3


Control
LY 215490 (i. t.)
0.1 µg
1 µg
10 µg
30 µg
Stim.
100µV
50ms

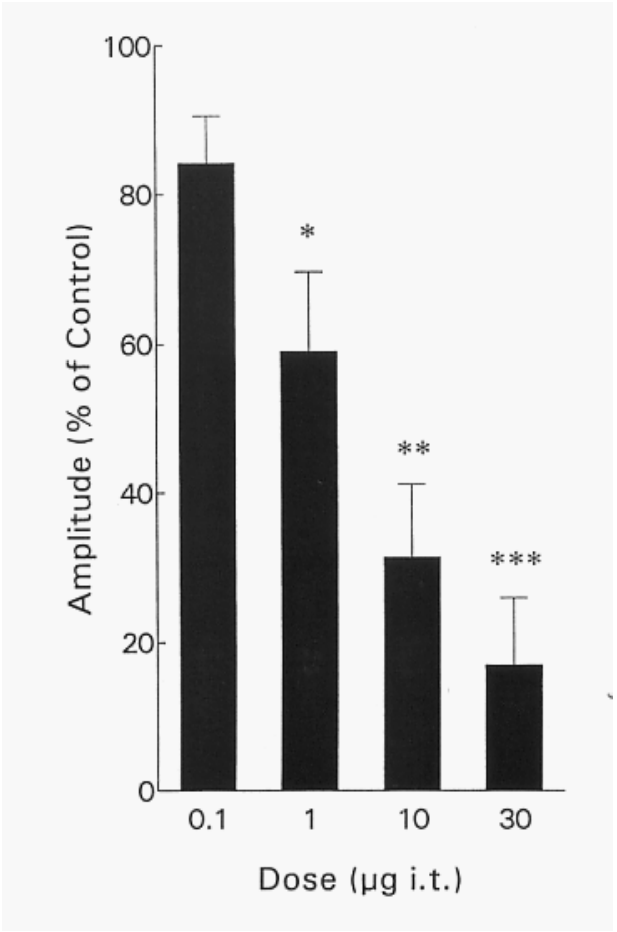

Amplitude (% of Control)
100
80
60
40
20
0
*
**
***
0.1
1
10
30
Dose (µg i.t.)

Figure 4

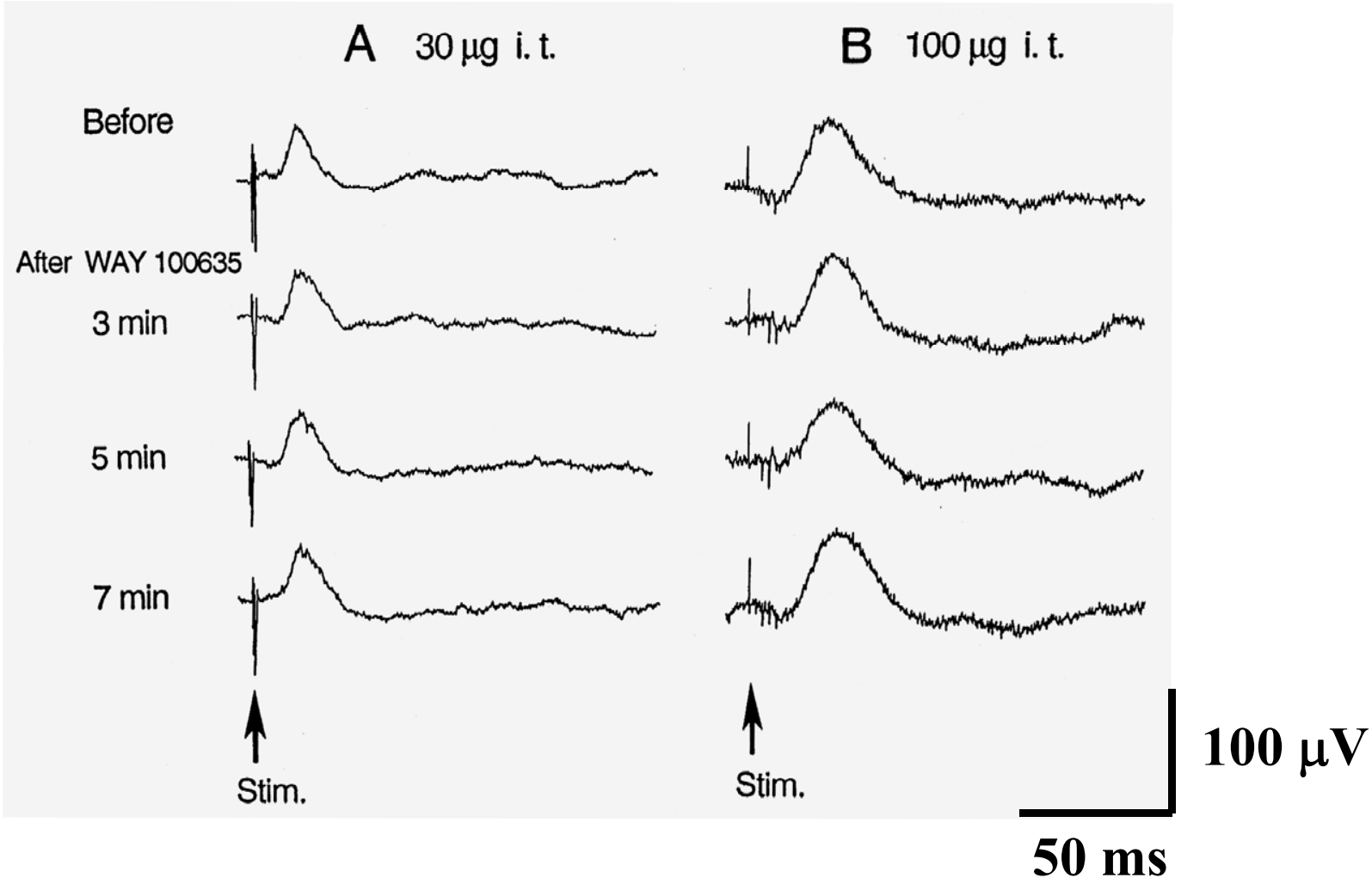

A 30 µg i. t.
B 100 µg i. t.
Before
After WAY 100635
3 min
5 min
7 min
Stim.
Stim.
100 µV
50 ms

Figure 5

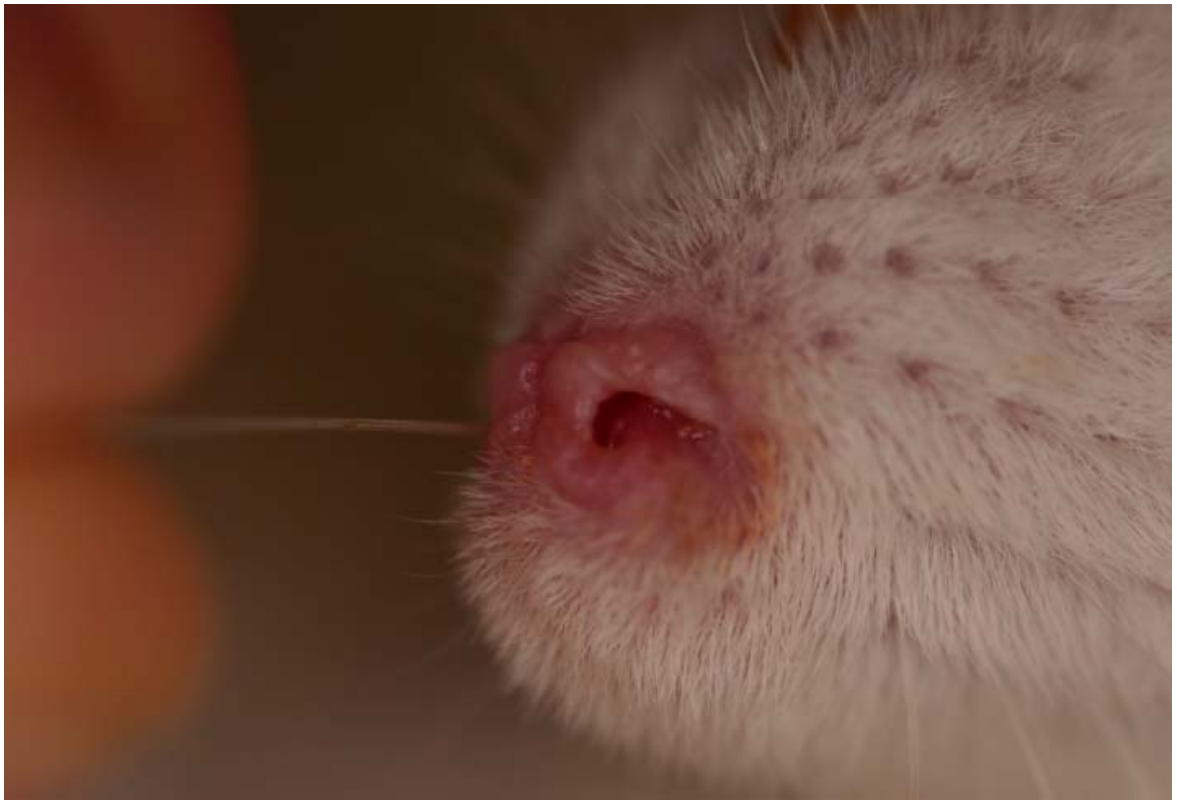
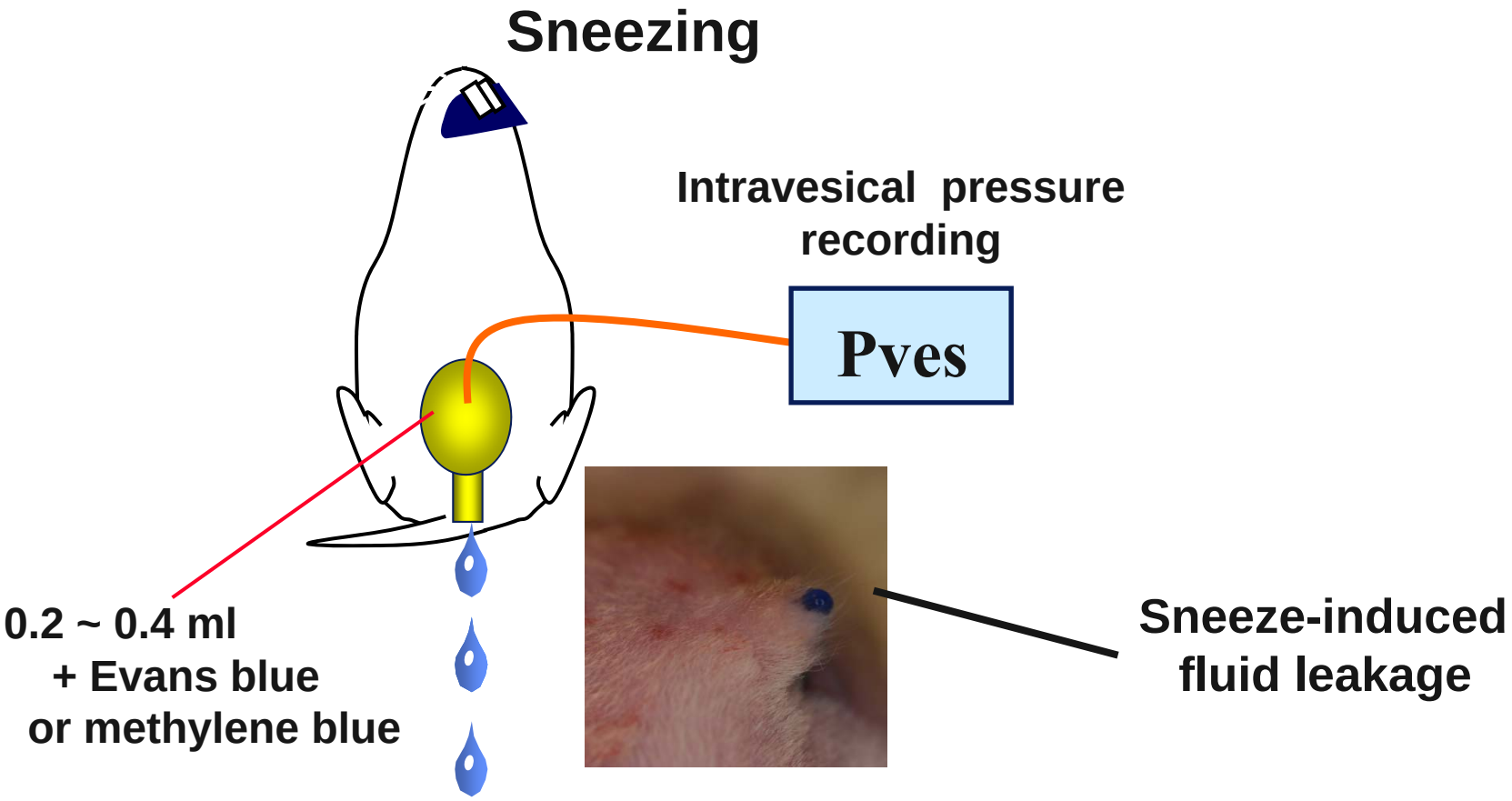


Figure 6


Sneezing
Intravesical pressure recording
Pves
0.2 ~ 0.4 ml
+ Evans blue
or methylene blue
Sneeze-induced fluid leakage