Inhibitory effect of the nucleus reticularis pontis oralis on the pontine micturition center and pontine urine storage center in decerebrate cats

Kimio SUGAYA, Saori NISHIJIMA, Minoru MIYAZATO, Masami ODA and Yoshihide OGAWA

From the Division of Urology, Department of Organ-oriented Medicine, Faculty of Medicine, University of the Ryukyus, Okinawa

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ABSTRACT

The influence of the nucleus reticularis pontis oralis (PoO) on the pontine micturition center (PMC) and pontine urine storage center (PUSC) was examined in decerebrate cats by electrical and chemical stimulations of the PMC, PUSC or PoO. Microinjection of carbachol into the rostral and dorsolateral part of the PoO rapidly inhibited reflex micturition and external urethral sphincter (EUS) activity. After confirming the inhibition of reflex micturition and EUS activity by microinjection of carbachol into the PoO, intravenous injection of atropine sulfate or its microinjection into the PoO recovered both reflex micturition and EUS activity. Microinjection of carbachol into the PMC evoked micturition and then inhibited reflex micturition, but intravenous injection of atropine or its microinjection into the PoO recovered reflex micturition. After confirming the inhibition of reflex micturition and EUS activity by microinjection of carbachol into the PoO, electrical stimulation of the PUSC enhanced EUS activity, but electrical stimulation of the PMC failed to evoke micturition. However, electrical stimulation of the PMC evoked micturition after microinjection of atropine into the PoO. These results suggest that the PoO strongly inhibits the PMC and less strongly inhibits the PUSC. Therefore, the PoO seems to be the pontine micturition inhibitory area.

The pontine micturition center (PMC) is the site where electrical stimulation evokes micturition, and it corresponds to the nucleus locus coeruleus alpha (LCa) in cats (16) and dogs (13). Electrical stimulation of the region ventrolateral to the PMC enhances external urethral sphincter (EUS) activity and inhibits bladder contraction (2, 6, 12). This region is called the pontine urine storage center (PUSC) or L-region, and it corresponds to the nucleus locus subcoeruleus (LSC) in cats (2, 6) and dogs (12). The third pontine region controlling lower urinary

tract function is located at a ventromedial site to the locus coeruleus complex. Electrical stimulation or microinjection of carbachol (a long-acting cholinomimetic agent that is resistant to acetylcholinesterase) into the third pontine region inhibits both bladder contraction and EUS activity (3, 17), and this region corresponds to the nucleus reticularis pontis oralis (PoO). While, microinjection of flavoxate hydrochloride into the PoO only inhibits bladder contraction without affecting EUS activity (3). Thus, the influence of the PoO on EUS activity was uncertain.

In this study using cats, we examined the relationship among the PMC, PUSC, and PoO with respect to bladder and EUS activity, especially the influence of the PoO on the function of the PMC and PUSC, by a combination of electrical and chemical stimulation of the PMC, PUSC, or PoO, as well as a urodynamic study that included cystome-

Address correspondence to: Kimio Sugaya, M. D.
Division of Urology, Department of Organ-oriented
Medicine, Faculty of Medicine, University of the
Ryukyus, 207 Uehara, Nishihara, Okinawa, 903-0215,
Japan
Tel: +81-98-895-1186, Fax: +81-98-895-1429
E-mail: sugaya@med.u-ryukyu.ac.jp

try and electromyography (EMG) of the EUS.

MATERIALS AND METHODS

A total of 38 adult cats of either sex weighing 2.0 to 4.1 kg were used in this study. Under anesthesia with halothane and nitrous oxide, a tube was inserted into the trachea after tracheostomy and the bilateral carotid arteries were tied. In order to perform cystometry with rapid drip-infusion of physiological saline into the bladder, and to avoid the influence of such infusion on the recorded bladder pressure, two catheters (6 Fr) were inserted together through the bladder dome (16, 17). The first catheter was used to record pressure data and the other was employed to fill the bladder with physiological saline. Bipolar electrodes made of thin (50 μ m) stainless-steel wire were directly inserted into the EUS around the urethra near the pelvic floor muscles using a fine needle for EMG. After craniotomy, a decerebrate state was created by transection at the precollicular-post-mammillary level (16, 17), and then anesthesia was discontinued. The head, thoracic vertebrae, and pelvis of the cat were fixed in a stereotaxic instrument. Spontaneous respiration was maintained before and after decerebration. Two hours after fixation in the apparatus, physiological saline was drip-infused into the bladder at 2–5 mL/min for cystometry.

To find and stimulate the PMC and PUSC, a glass micropipette filled with Woods metal was used, with the tip being replaced by a carbon fiber having a diameter of 7 μ m (resistance: 0.2 to 0.5 M Ω). This microelectrode was systematically inserted into the rostral pons (Horsley-Clarke coordinates, P 0 to 4, LR 0 to 5, H -1 to -5) using a micromanipulator. The PMC (P 2.5 to 3.0, LR 2.5 to 2.75, H -2.25 to -2.75) was identified as the site where micturition was elicited by electrical stimulation at 20 μ A (pulse duration of 0.2 ms, intratrain frequency of 50 Hz, and train duration of 3–5 s) with the bladder filled to 80% of the volume evoking reflex micturition. The PUSC (P 2.5 to 3.0, LR 3.0 to 3.5, H -2.75 to -3.5) was identified as the site where EMG activity of the EUS was promoted by electrical stimulation at 20–50 μ A (pulse duration of 0.2 ms and intratrain frequency of 50 Hz) without bladder contraction and asymmetrical movement of the hind limbs. For microinjection of carbachol, a glass microcapillary (tip diameter; 20 μ m) filled with 0.1 M carbachol (carbamylcholine chloride; Sigma, St. Louis, USA) dissolved in 0.1 M phosphate-buffered saline (pH 7.4) was employed. It was attached to the stimulating platinum-iridium wire electrode. Since the PoO is

relatively large, in order to find the optimal site where microinjection of carbachol inhibited reflex micturition most rapidly, the glass microcapillary filled with carbachol was inserted into various sites in the rostral pons (P 1.0 to 4.0, LR 0.5 to 3.5, H -2.0 to -5.0), and carbachol (0.5 μ L) was injected focally for 10–20 s using a micropressure pump ($n = 19$). The latency between carbachol microinjection into the pons and the inhibition of reflex micturition was examined by repeating cystometry at 1-min intervals, with saline being infused rapidly (20–40 mL/min) to evoke reflex micturition within 1 min after the start of infusion. Infusion of saline was immediately stopped when micturition or overflow incontinence occurred. Then, rapid infusion of saline into the bladder was restarted after micturition.

Atropine sulfate (0.05% solution; Tanabe, Osaka, Japan), a muscarinic receptor antagonist, was also administered by intravenous injection (0.5–1.0 mg/1–2 mL) or microinjection into the PoO (0.5–1.0 μ g/1–2 μ L) using a glass microcapillary after the microinjection of carbachol to examine whether it could block the effect of carbachol. Other cats received carbachol injection into the PMC, followed by injection of atropine sulfate intravenously or microinjection into the PoO to examine the influence of carbachol on the PMC and its diffusion to the PoO.

The effects of electrical stimulation of the PMC or PUSC were also examined before and after microinjection of carbachol into the optimal PoO site, as well as after intravenous injection of atropine sulfate or its microinjection into the PoO to examine the functional relationship between the PoO and PMC or PUSC. The bladder pressure and EUS-EMG activity were recorded on a paper recorder during cystometry.

The site used for electrical stimulation of the PMC and PUSC, and the locations of carbachol microinjection, were marked with electrolytic microlesions by passing a cathodal direct current (30 μ A) through the electrode for 30 s. Then each animal was sacrificed under deep sodium pentobarbital anesthesia (50 mg/kg intraperitoneally), and the brainstem was removed and fixed in 0.9% saline containing 10% formalin. Frontal plane frozen sections (50 μ m) were cut and stained with neutral red, and the locations of the microlesions were identified with reference to the stereotaxic atlases of Berman (1), Taber (21), and Maeda *et al.* (7).

RESULTS

Identification of the rostral pontine region where carbachol most rapidly inhibited reflex micturition (n=19)

Rapid infusion of saline into the bladder evoked reflex micturition at a threshold volume of 15–34 mL and a bladder contraction pressure of 38–55 cm H₂O. When reflex micturition was repeatedly evoked at 1-min intervals by rapid infusion of saline into the bladder, microinjection of carbachol into the PoO or its surroundings inhibited such reflex micturition (Figs. 1A, 1B). Microinjection of carbachol into the rostral and dorsolateral part of the PoO (P2–P3, LR1.75, and H–0.35), which is close to the LCa, rapidly inhibited (within 1 min) EUS-EMG activity and reflex micturition, after which the bladder pressure gradually increased until overflow incontinence occurred at a pressure of 25–56 cm H₂O (Fig. 1C). When carbachol microinjection was done adjacent to this region, the time longer than 1 min was required to inhibit reflex micturition. Inhibition

of reflex micturition persisted for about 2–3 h after carbachol microinjection. Therefore, we selected the rostral and dorsolateral part of the PoO (P2.5, LR1.75, and H–0.35) as the site for carbachol microinjection in the following experiments.

Carbachol microinjection into the PoO, followed by intravenous injection of atropine sulfate or its microinjection into the PoO (n=6)

After the occurrence of reflex micturition had been confirmed, the bladder was infused with a subcritical (80%) volume of saline. Microinjection of carbachol into the PoO inhibited EUS-EMG activity. Further infusion of saline induced overflow incontinence without bladder contraction when the total volume infused reached 1.6–2.5 times that inducing reflex micturition. The bladder was drained after 30 min, and the intravenous injection of atropine sulfate (n=3) or its microinjection into the PoO (n=3) recovered reflex micturition at almost same volume of saline as that inducing micturition before carbachol injection (Figs. 2A, 2B). After the recov-

Fig. 1 Site where carbachol microinjection into the rostral pons inhibits reflex micturition most effectively. A: Reflex micturition evoked by rapid infusion of saline into the bladder. B: Inhibition of reflex micturition by carbachol microinjection into the PoO. Microinjection of carbachol inhibited EUS-EMG activity and overflow incontinence occurred when saline was rapidly infused into the bladder. C: Site of carbachol microinjection and latency between carbachol injection and the inhibition of reflex micturition. After carbachol microinjection into the rostral and dorsolateral part of the PoO (P2–P3, LR1.75, and H–0.35), which is close to the LCa, the suppression of reflex micturition was most rapid (within 1 min). Carb.: carbachol, inj.: microinjection.

Fig. 2 Inhibitory effect of carbachol microinjection into the PoO on lower urinary tract function and its recovery by atropine sulfate. Reflex micturition was evoked when 20 mL (A) or 30 mL (B) of saline was infused into the bladder. When the bladder was infused with 16 mL (A) or 24 mL (B) of saline, microinjection of carbachol into the PoO inhibited EUS-EMG activity. Additional infusion of saline into the bladder induced overflow incontinence without bladder contraction when the total volume of saline in the bladder reached 50 mL (A) or 58 mL (B). The bladder was drained after 30 min, following which intravenous injection of 1 mg of atropine sulfate (A) or microinjection of 0.5 μ g of atropine sulfate into the PoO (B) recovered the reflex micturition response to 20 mL (A) or 27 mL (B) of saline. After the return of reflex micturition, EUS-EMG activity also recovered. BC: bladder capacity, iv.: intravenous injection.

ery of reflex micturition, EUS-EMG activity also recovered.

Carbachol microinjection into the PMC, followed by intravenous injection of atropine sulfate or its microinjection into the PoO (n=6)

After the occurrence of reflex micturition had been confirmed, the bladder was infused with a subcritical (80%) volume of saline. Microinjection of carbachol into the PMC evoked micturition. Subsequently, reflex micturition was inhibited and overflow incontinence occurred around the same volume of saline as after carbachol microinjection into the PoO. The bladder was drained after 30 min, and the intravenous injection of atropine sulfate (n = 3) or its microinjection into the PoO (n = 3) recovered reflex micturition at almost same volume of saline as that inducing micturition before carbachol microinjection into the PMC (Figs. 3A, 3B).

Electrical stimulation of the PMC before and after microinjection of carbachol and atropine sulfate into the PoO (n=3)

After reflex micturition was confirmed, the bladder was infused with a subcritical (80%) volume of saline. Electrical stimulation of the PMC was demonstrated to evoke micturition. Subsequent microinjection of carbachol into the PoO inhibited reflex micturition and EUS-EMG activity. After 30 min,

electrical stimulation of the PMC failed to evoke micturition when the bladder was infused with a subcritical (80%) volume of saline, even when the stimulus intensity was raised three-fold from 20 μ A to 60 μ A. However, electrical stimulation of the PMC evoked micturition after microinjection of atropine sulfate into the PoO (Fig. 4). Following the recovery of micturition evoked by PMC stimulation, EUS-EMG activity also recovered.

Carbachol microinjection into the PoO, followed by electrical stimulation of the PMC and PUSC (n=4)

After reflex micturition was confirmed, the bladder was infused with a subcritical (80%) volume of saline. Electrical stimulation of the PMC was demonstrated to evoke micturition. Then microinjection of carbachol into the PoO was performed. After 30 to 60 min, EUS-EMG activity recovered slightly when the bladder was infused with a subcritical (80%) volume of saline. At that time, electrical stimulation of the PMC did not induce any changes of bladder pressure or EUS-EMG activity (Fig. 5A), but electrical stimulation of the PUSC enhanced EUS-EMG activity (Fig. 5B).

DISCUSSION

This study showed that neurons in both the PMC and PoO possess cholinergic receptors, and that cho-

Fig. 3 Effect of carbachol microinjection into the PMC on lower urinary tract function and its recovery by atropine sulfate. Reflex micturition was evoked when 20 mL (A) or 25 mL (B) of saline was infused into the bladder. When the bladder was infused with 16 mL (A) or 20 mL (B) of saline, microinjection of carbachol into the PMC evoked micturition. Further infusion of saline induced overflow incontinence when the total volume infused into the bladder reached 50 mL (A) or 60 mL (B), and EUS-EMG activity was also inhibited. The bladder was drained after 30 min, following which intravenous injection of 1 mg of atropine sulfate (A) or microinjection of 0.5 μ g of atropine sulfate (B) recovered the reflex micturition with 20 mL of saline (A and B). After recovery of reflex micturition, EUS-EMG activity also recovered.

Fig. 4 Effect of electrical stimulation of the PMC before and after microinjection of carbachol and atropine sulfate into the PoO. When the bladder was infused with a subcritical volume (20 mL) of saline, electrical stimulation (20 μ A) of the PMC evoked reflex micturition. Subsequent microinjection of carbachol into the PoO inhibited both reflex micturition and EUS-EMG activity. While the bladder was infused with a subcritical volume (20 mL) of saline at 28 min later, electrical stimulation of the PMC failed to evoke micturition even at a stimulus intensity of 60 μ A. After microinjection of 0.5 μ g of atropine sulfate into the PoO, however, electrical stimulation of the PMC at 20 μ A evoked micturition (Fig. 4). Following the recovery of micturition evoked by PMC stimulation, EUS-EMG activity also recovered. Stim.: stimulation.

linergic stimulation of the PMC and PoO evokes and inhibits micturition, respectively. The neurons in the PMC were atropine-resistant, while those in the PoO were atropine-sensitive, suggesting that the PMC cells have nicotinic receptors and the PoO cells have muscarinic receptors. After bladder and EUS-EMG activities were inhibited by microinjection of carbachol into the PoO, electrical stimulation of the PMC was unable to induce bladder contraction, but electrical stimulation of the PUSC promoted EUS-EMG activity. These results suggested that the PoO strongly inhibits the activity of the PMC, while it also inhibits the PUSC, but not so strongly. Taken together, these results indicated that the PoO may represent the pontine micturition inhibitory area

(20).

Since carbachol is not metabolized by acetylcholinesterases, its microinjection causes not only an effect at the injection site but also other effects due to diffusion into surrounding structures. Microinjection of carbachol into the PMC initially evoked micturition and then inhibited reflex micturition, while microinjection of atropine sulfate into the PoO recovered reflex micturition, suggesting that carbachol has diffused into the PoO from the PMC. The site in the PoO where carbachol microinjection most rapidly inhibited reflex micturition was the same as that where electrical stimulation or microinjection of flavoxate hydrochloride have been reported to inhibit reflex micturition (3, 4), *i. e.*, the rostral and dor-

Fig. 5 Effect of electrical stimulation of the PMC or PUSC after microinjection of carbachol into the PoO. At 50 min after microinjection of carbachol into the PoO, EUS-EMG activity showed slight recovery when the bladder was infused with a subcritical volume of saline. At that time, electrical stimulation of the PMC (50 μ A) failed to induce any changes of bladder pressure on cystometry (CMG) or EUS-EMG activity (A), but electrical stimulation of the PUSC (50 μ A) enhanced EUS-EMG activity (B).

solateral part of the PoO (P2–P3, LR1.75, and H –0.35) located close to the LCa. Therefore, it is possible that many of the neurons controlling micturition reside in this part of the PoO.

The descending pathways from the PoO project to the spinal cord either directly or via the nucleus reticularis gigantocellularis in the medulla oblongata (8, 20, 22). Microinjection of carbachol into the rostral pontine reticular formation in rats, corresponding to the PoO in cats, inhibits bladder contraction and induces an increase of glycine level in the lumbosacral cord (11). Spinal glycinergic neurons are major inhibitory interneurons involved in the control of lower urinary tract function (9, 10). Therefore, the inhibitory effects of the PoO may be mediated via spinal inhibitory glycinergic interneurons. Since the motoneurons in Onuf's nucleus, which innervate the EUS, receive projection from glycinergic and glycinergic/GABAergic interneurons in the lumbosacral cord (15), activation of the PoO may also inhibit EUS activity via these inhibitory interneurons. Electrical stimulation of the PUSC induced EUS-EMG activity after microinjection of carbachol into the PoO had inhibited PMC function, suggesting that descending excitatory projections from the PUSC to Onuf's nucleus were stronger than the inhibitory projections from the PoO via glycinergic interneurons. The excitatory projections from the PUSC to Onuf's nucleus are thought to be glutamatergic, because intrathecal injection of glutamate receptor antagonists inhibits EUS activity more than bladder contraction (14, 24). Therefore, it may be possible that a functional relationship between the PoO and PUSC at least exists at the level of the

sacral cord. Although neuroanatomical studies using neurotracers have shown that there are connections among the PMC (LCa), PUSC (LSC), and PoO, there are rather sparse (5, 18, 19). Therefore, the functional relationship among these three areas is thought to be weak at the level of the pons. Since microinjection of carbachol into the dorsolateral pontine area induces muscle atonia and rapid eye movement (REM) sleep (8, 22, 23), bladder contraction may be also inhibited during REM sleep. However, EUS tone may be maintained during REM sleep to prevent urinary incontinence while sleeping.

In conclusion, this study demonstrated that the PoO may be the pontine micturition inhibitory area, inhibiting both bladder contraction and EUS activity, although the PoO does not completely control the PUSC.

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REFERENCES

1. Berman AL (1968) *The Brain Stem of the Cat. A Cytoarchitectonic Atlas with Stereotaxic Coordinates*. The University Wisconsin Press, Wisconsin.
2. Holstege G, Griffiths D, de Wall H and Dalm E (1986) Anatomical and physiological observations on supraspinal control of bladder and urethral sphincter muscles in the cat. *J Comp Neurol* **250**, 449–461.
3. Kimura Y, Ukai Y, Kimura K, Sugaya K and Nishizawa O (1995) Inhibitory influence from the nucleus reticularis pontis oralis on the micturition reflex induced by electrical stimulation of the pontine micturition center in cats. *Neurosci Lett* **195**, 214–216.
4. Kimura Y, Sasaki Y, Hamada K, Fukui H, Ukai Y, Yoshikuni Y, Kimura K, Sugaya K and Nishizawa O (1996) Mechanisms of the suppression of the bladder activity by flavoxate. *Int J Urol* **3**, 218–227.
5. Kohama T (1992) Neuroanatomical studies on the urine storage facilitatory areas in the cat brain. Part 2. Output neuronal structures from the nucleus locus subcoeruleus and the nucleus reticularis pontis oralis. *Jpn J Urol* **83**, 1478–1483.
6. Kuru M (1965) Nervous control of micturition. *Physiol Rev* **45**, 425–494.
7. Maeda T, Pin C, Salvat D, Ligier M and Jouvet M (1973) Les neurones contenant des catécholamines du tegmentum pontique et leurs voies de projection chez le chat. *Brain Res* **57**, 119–152.
8. Mori S (1989) Contribution of postural muscle tone to full

- expression of posture and locomotor movements: Multi-faceted analyses of its setting brainstem-spinal cord mechanisms in the cat. *Jpn J Physiol* **39**, 785–809.
9. Nishijima S, Sugaya K, Miyazato M, Ashitomi K, Morozumi M and Ogawa Y (2003) Changes of bladder activity and glycine levels in the spinal cord and serum after spinal cord injury in rats. *Biomed Res* **24**, 165–171.
 10. Nishijima S, Sugaya K, Miyazato M, Ashitomi K, Morozumi M and Ogawa Y (2003) Relationship between bladder activity and amino acid levels in the central nervous system in rats with cerebral infarction. *Biomed Res* **24**, 173–180.
 11. Nishijima S, Sugaya K, Miyazato M, Shimabukuro S, Morozumi M and Ogawa Y (2005) Activation of the rostral pontine reticular formation increases the spinal glycine level and inhibits bladder contraction in rats. *J Urol* **173**, 1812–1816.
 12. Nishizawa O, Sugaya K, Noto H, Harada T and Tsuchida S (1987) Pontine urine storage center in the dog. *Tohoku J Exp Med* **153**, 77–78.
 13. Nishizawa O, Sugaya K, Noto H, Harada T and Tsuchida S (1988) Pontine micturition center in the dog. *J Urol* **140**, 872–874.
 14. Nishizawa O, Igawa Y, Satoh T, Yamashiro S and Sugaya K (1999) Effects of glutamate receptor antagonists on lower urinary tract function in conscious unanesthetized rats. *Adv Exp Med Biol* **462**, 275–281.
 15. Shefchyk SJ (2006) Spinal mechanisms contributing to urethral striated sphincter control during continence and micturition: “how good things might go bad”. *Prog Brain Res* **152**, 85–95.
 16. Sugaya K, Matsuyama K, Takakusaki K and Mori S (1987) Electrical and chemical stimulations of the pontine micturition center. *Neurosci Lett* **80**, 197–201.
 17. Sugaya K, Mori S, Nishizawa O, Noto H and Tsuchida S (1987) Chemical stimulations of the pontine micturition center and the nucleus reticularis pontis oralis. *Neurol Uro-dyn* **6**, 143–144.
 18. Sugaya K, Mori S and Tsuchida S (1988) Input and output neuronal structures of the pontine micturition center. Part 1. Mainly input neuronal structures. *Jpn J Urol* **79**, 1210–1218.
 19. Sugaya K, Mori S and Tsuchida S (1988) Input and output neuronal structures of the pontine micturition center. Part 2. Mainly output neuronal structures. *Jpn J Urol* **79**, 1219–1227.
 20. Sugaya K, Nishijima S, Miyazato M and Ogawa Y (2005) Central nervous control of micturition and urine storage. *J Smooth Muscle Res* **41**, 117–132.
 21. Taber E (1971) The cytoarchitecture of the brain stem of the cat. I. Brain stem nuclei of cat. *J Comp Neurol* **116**, 27–70.
 22. Takakusaki K, Shimoda N, Matsuyama K and Mori S (1994) Discharge properties of medullary reticulospinal neurons during postural changes induced by intrapontine injections of carbachol, atropine and serotonin, and their functional linkages to hindlimb motoneurons in cats. *Exp Brain Res* **99**, 361–374.
 23. Xi MC, Morales FR and Chase MH (2004) Interactions between GABAergic and cholinergic processes in the nucleus pontis oralis: neuronal mechanisms controlling active (rapid eye movement) sleep and wakefulness. *J Neurosci* **24**, 10670–10678.
 24. Yoshiyama M, Roppolo JR and de Groat WC (1997) Effects of LY215490, a competitive alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonist, on the micturition reflex in the rat. *J Pharmacol Exp Ther*, **280**, 894–904.