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

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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 (LCo) 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 coerules 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-
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 μm) 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-postmammillary 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 μm (resistance: 0.2 to 0.5 MΩ). 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 μA (pulse duration of 0.2 ms, intraintrain 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 μA (pulse duration of 0.2 ms and infraintrain frequency of 50 Hz) without bladder contraction and asymmetrical movement of the hind limbs. For microinjection of carbachol, a glass microcapillary (tip diameter; 20 μm) 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 μL) 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 μg/1–2 μL) 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 μA) 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 μm) 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-
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 PoO inhibited EUS-EMG activity. 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 μA to 60 μA. However, electrical stimulation of thePMC 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-
The pontine micturition inhibitory area

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-
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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