Micturition-suppressing region in the periaqueductal gray of the mesencephalon of the cat

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Holstege et al. (10) reported a region in the lateral pontine micturition-center (PMC; 19). On the other hand, we identified a region in the pontine tegmentum that induced an increase in internal urethral pressure or partial bladder contractions. This region was called M-region (10) or the pontine micturition-center (PMC) induced micturition region in the PAG. These results suggest that the dorsolateral margin of the rostral PAG includes the micturition-suppressing region that seems to have neural connections with the PMC. GABA is assumed to be one of the neurotransmitters that are involved in the PMC inhibition from the micturition-suppressing region in the PAG.

MATERIALS AND METHODS

In this study, we used 27 adult cats (15 male and 12 female) weighing between 2.4 and 4.8 kg. The animal protocols were conducted in accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals” and guidelines on the ethical use of animals in Asahikawa Medical College. This study was approved by Asahikawa Medical College Animal Care Committee. All efforts were made to minimize the number of animals used and their suffering.

A tracheotomy was performed under general anesthesia with halothane inhalation. The unilateral carotid artery was cannulated for recording blood pressure, and the contralateral carotid artery was ligated to minimize bleeding at the time of decerebration. The femoral vein was cannulated for a supplementary infusion of physiological saline. With a lower abdominal midline incision, a 6-French double-lumen catheter was inserted into the bladder through the urethra for monitoring intravesical pressure and for bladder filling. From the same abdominal incision, two stainless steel wire electrodes were inserted in the external urethral sphincter for recording electromyography (EMG). Craniotomy was performed, and the brain was decerebrated at the supracollicular postmamillary level. The cerebellum was exposed after removing the osseous cerebellar tentorium. Anesthesia was discontinued after decerebration, and the cats were fixed on a stereotaxic puncture apparatus (Narishige, Tokyo, Japan), and the cats were fixed on a stereotaxic puncture apparatus (Narishige, Tokyo, Japan), and the cats were fixed on a stereotaxic puncture apparatus (Narishige, Tokyo, Japan). Holstege et al. (10) reported a region in the lateral pontine tegmentum that induced an increase in internal urethral pressure by electrical stimulation and named it L-region as the pontine ureter storage center. Thus, the pontine tegmentum is critical for micturition and urine storage. Recent studies in cats and rats found that electrical and chemical stimulation of the ventrolateral periaqueductal gray (PAG) induced micturition (15, 20). These results implicate an important role of the mesencephalon in the micturition reflex.

The mesencephalon is involved in the integration and regulation of the cardiovascular and respiratory system, body temperature, vocalization, pain and analgesia, defensive behaviors, and lordosis (4, 23). Several studies have revealed that neurons ascending from the sacral spinal cord have more neural communications with the PAG than with the PMC (6, 8, 21). Recent studies with positron emission tomography in humans showed an increase in regional cerebral blood flow in the PAG during urine storage (2, 16). Therefore, we hypothesized that there would be a micturition-suppressing region in the PAG. We investigated the existence of micturition-suppressing region in the PAG and its possible neurotransmitter in cats.

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dehyde, and 40-μm frozen slices were prepared to detect stimulation sites.

**Neurotransmitter.** Since GABA has been well known as a widely distributed neurotransmitter in the central nervous system and as densely distributed around the locus coeruleus (1), we focused on a possible involvement of GABA in the PMC suppression from the micturition-suppressing region in the PAG. After identifying the most effective site for suppressing spontaneous bladder contractions by electrical stimulation of the PAG, we reduced the saline volume in the bladder to 5 ml for suppressing spontaneous bladder contractions. We then identified a micturition-inducing site at the pontine tegmentum (PMC) by weak intensity (20–40 μA) electrical stimulation with an insulated stainless steel wire electrode that was attached to a glass micropipette (50 μm). While stimulating the PMC and micturition-suppressing region in the PAG simultaneously, we injected the GABA body blocker, bicuculline methiodide (2 mM, 0.1 to 0.4 μl; Sigma, St. Louis, MO), into the PMC through the micropipette and continued to measure intravesical pressure to investigate whether GABA is one of the neurotransmitters that are involved in the inhibitory pathway from micturition-suppressing region in the PAG to the PMC.

**RESULTS**

**Electrical stimulation.** Spontaneous isovolumetric bladder contractions occurred during 1 to 2 h after decerebration. The duration of each bladder contraction was 32 to 55 s, and the maximum pressure of isovolumetric bladder contractions ranged from 30 to 93 cmH2O (Fig. 1A). Bladder contraction resumed 23 to 25 s after the cessation of previous bladder contraction. During bladder contractions, EMG activity of the external urethral sphincter decreased (Fig. 1A), which is consistent with reciprocal activity of the bladder and sphincter in the cat.

Micturition-suppressing sites in the PAG were identified as those that suppressed bladder contractions almost completely (Fig. 1B). Intravesical pressure was reduced to the almost baseline level by electrical stimulation of micturition-suppressing region. EMG activity of the external urethral sphincter did not increase during electrical stimulation of the micturition-suppressing region (Fig. 1B). After the cessation of electrical stimulation, EMG activity of the external urethral sphincter increased. Bladder contractions resumed 14 to 18 s after the cessation of electrical stimulation. Regarding blood pressure change, blood pressure was slightly elevated during spontaneous isovolumetric bladder contractions. Blood pressure was significantly elevated with electrical stimulation of the micturition-suppressing region (Fig. 1B). Blood pressure and respiratory responses were clearly related to electrical stimulation (Fig. 1B). Because bladder contractions were suppressed by 40 μA but not by 20 μA of electrical stimulation (data not shown), the threshold current for suppressing bladder contractions was estimated to be 40 μA. Figure 2 shows examples of the tracks of the microelectrode insertion. The location of the most effective micturition-suppressing site was restricted, while adjacent sites also suppressed bladder contractions if stimulated with higher current (80 μA). The most effective micturition-suppressing sites were all ablated with direct electrical current for marking the location (Fig. 3, inset). One to three sites were recorded in each cat, and thus we collected a total of 34 sites (Fig. 3). The most effective micturition-

**Fig. 1.** Bladder pressure, blood pressure, respiration, and external urethral sphincter electromyography (sphincter EMG) traces before (A) and during (B) electrical stimulation of the micturition-suppressing site in the periaqueductal gray (PAG). Arrows indicate beginning of isovolumetric bladder contractions. A: spontaneous isovolumetric bladder contractions occurred during 1 to 2 h after decerebration. EMG activity of the external urethral sphincter decreased during bladder contractions. EMG activity increased after the end of bladder contractions. B: bladder contractions were suppressed by electrical stimulation. Transient blood pressure increase was synchronized with stimulation. EMG activity did not increase during electrical stimulation of the micturition-suppressing site.

**Fig. 2.** Examples of the location of the most effective micturition-suppressing site in the PAG where electrical stimulation with 40 μA current suppressed bladder contractions (black dots). Superficial or deeper sites in the same tract could suppress bladder contractions only if higher current (80 μA) was used for electrical stimulation (gray dots). White circles indicate ineffective sites where electrical stimulation produced no effect on bladder contractions. SC, superior colliculus; IC, inferior colliculus.

**Horsley-Clarke axis**

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**Mesencephalon**

- Most effective micturition suppressing site
- Micturition suppressing site
- Ineffective site

**Fig. 3.** Examples of the location of the most effective micturition-suppressing site in the PAG where electrical stimulation with 40 μA current suppressed bladder contractions (black dots). Superficial or deeper sites in the same tract could suppress bladder contractions only if higher current (80 μA) was used for electrical stimulation (gray dots). White circles indicate ineffective sites where electrical stimulation produced no effect on bladder contractions. SC, superior colliculus; IC, inferior colliculus.
suppressing sites were detected at the rostral portion of the dorsolateral PAG (P1-A3, L2-L3, H3.5–H1.5; Fig. 3).

**Neurotransmitter.** Study of the neurotransmitter was performed in six cats. Bladder contractions were provoked by electrical stimulation of the PMC (bladder contraction pressure between 40 and 80 cmH\textsubscript{2}O; Fig. 4). Then, simultaneous electrical stimulation was applied to the PMC and micturition-suppressing region in the PAG. No bladder contractions were provoked (3 cats; Fig. 4), or only weak bladder contractions were provoked (3 cats) by simultaneous electrical stimulation of the both regions. After a microinjection of bicuculline into the PMC, simultaneous electrical stimulation of the both regions induced bladder contractions with pressure between 20 and 40 cmH\textsubscript{2}O (Fig. 4).

**DISCUSSION**

The present study showed the existence of micturition-suppressing sites in the dorsolateral margin of the rostral PAG that inhibited bladder contractions without increasing activity of the external urethral sphincter. Neurotransmitter study suggests that GABA is assumed to be one of neurotransmitters that are involved in the PMC inhibition from the micturition-suppressing region in the PAG.

The PMC in the cat is located ventral to the locus coeruleus and the mesencephalic trigeminal tracts in the tegmental pons (12), while the PMC in the rat is recognized at the dorsolateral tegmental nucleus (7, 18). Anatomical location of the PMC is different between the cat and rat. The PMC projects directly to the dorsal gray commissure and intermediomedial cell groups of the spinal cord. Excitation of the PMC induces bladder contraction via parasympathetic preganglionic neurons at the intermediomedial cell groups and simultaneous relaxation of the external urethral sphincter by suppressing the nucleus of Onuf via interneurons at the dorsal gray commissure. Thus the PMC activation is critical for inducing normal micturition. The PMC receives descending control from the PAG. Blok and Holstege (5) reported direct projections from the PAG to the PMC. Taniguchi et al. (20) and Matsuura et al. (15) reported micturition-inducing sites in the PAG. In the latter two reports, micturition-inducing sites in the PAG were located at the ventrolateral portion of the caudal PAG.

Regarding urine storage, a micturition-suppressing region was found in the substantia nigra (22) and the basal ganglia (13). Several investigators reported a region at the tegmental pons that projected to the nucleus of Onuf and increased the external urethral sphincter activity and called it L-region or the pontine urine storage center (10, 17). A recent report by Liu et al. (14) showed a micturition-suppressing region at the ventral PAG. The present study identified a micturition-suppressing region at the dorsolateral portion of the rostral PAG. In the present study, electrical stimulation of the micturition-suppressing region did not increase EMG activity of the external urethral sphincter. Therefore, it seems that the function of the micturition-suppressing region in the PAG is independent of the pontine urine storage center. Compared with the micturition-inducing sites in the PAG (20), micturition-suppressing sites in the PAG were located more dorsal and rostral. In the present study, we could not induce bladder contractions
by electrical stimulation of the rostral PAG. Although slight overlapping might exist, micturition-suppressing sites in the PAG have different topographical localizations from micturition-inducing sites in the PAG.

In the present study, blood pressure increased simultaneously with electrical stimulation of the micturition-suppressing region in the PAG. Respiratory changes were also elicited by electrical stimulation. Blood pressure increase was previously observed by electrical stimulation of the PMC and micturition-inducing sites in the PAG (20). Weak-intensity electrical stimulation of the PMC elicited predominantly bladder contraction rather than blood pressure increase, while weak-intensity electrical stimulation of micturition-inducing sites in the PAG caused opposite response (20). Thus similar but different reactions of the bladder and blood pressure were observed during electrical stimulation of the PMC and PAG. Because the mesencephalon is involved in the integration and regulation of the cardiovascular and respiratory system (4, 23), electrical stimulation of different regions in the PAG can result in the same response of blood pressure.

We examined a possible involvement of GABA as a neurotransmitter from the PAG to the PMC that is distributed widely in the central nervous system (1). A recent study with electron microscopy indicated prominent GABAergic projections to the soma around the locus coeruleus in rats (1). In the present study, bladder contractions evoked by electrical stimulation of the PMC were completely suppressed by simultaneous electrical stimulation of the micturition-suppressing region in the PAG. After an injection of bicuculline into the PMC, bladder contractions were partially recovered. These findings suggest that GABA is one of neurotransmitters that are involved in the PMC inhibition from the micturition-suppressing region in the PAG. Injection of bicuculline into the PMC did not fully recover the PMC-evoked bladder contractions that were suppressed by simultaneous electrical stimulation of the PAG. The reason may be an insufficient concentration of bicuculline used in the present study or an involvement of synaptic receptors other than GABAA. Our previous study (20) revealed that the bilateral PMC had fiber communications each other. Thus the injection of bicuculline into the unilateral PMC might not have been sufficient for blocking the inhibitory action from the PAG. Because we did not examine direct neural connections between the micturition-suppressing region in the PAG and the PMC, there might be some possibility that GABA is not released from nerve terminals of the micturition-suppressing region in the PAG but from nerve terminals of intervening neurons. Neurotrace study is necessary to elucidate the precise neural connections between the micturition-suppressing region in the PAG and the PMC.

We identified micturition-suppressing regions in the PAG by electrical stimulation. Electrical stimulation can activate neurons as well as fibers of passage. We performed chemical stimulation of the most effective micturition-suppressing sites in the PAG by a microinjection of N-methyl-d-aspartate (NMDA). Microinjection of NMDA into the most effective micturition-suppressing site in the PAG suppressed bladder contractions completely in two cats and partially in another cat (data not shown). This suggests that micturition-suppressing sites in the PAG contained nerve cells. Taken together, we believe that the restricted region of the rostral PAG contains neurons that can suppress micturition. Further studies are necessary to clarify neurotransmitters and neural communications that are involved to connect micturition-suppressing region in the PAG and the PMC.

**Perspectives and Significance**

In the present study, bladder contractions were not suppressed but only reduced in amplitude by weak electrical stimulation (below 20 μA) of the most effective micturition-suppressing site in the PAG (data not shown). When the most effective micturition-suppressing site was fully stimulated (40 μA or higher for 10 s), bladder contractions were completely suppressed. Thus the inhibitory effect on the PMC is dependent on the degree of activation of the micturition-suppressing region in the PAG. This scenario might be applicable to patients with lower urinary tract dysfunction. People with normal lower urinary tract function usually maintain a low detrusor pressure even if they feel a strong desire to void. In patients with lower urinary tract dysfunction, we often see a phenomenon of involuntary detrusor contraction with various amplitude and duration (detrusor overactivity). In some patients detrusor overactivity is only phasic and associated with a slight increase in detrusor pressure, while in others detrusor overactivity is prominent and accompanied with incontinence. The severity of detrusor overactivity may correlate with the severity of dysfunction of the micturition-suppressing region in the PAG. It is tempting to speculate that a disorder of the micturition-suppressing region in the PAG may be one of the central mechanisms of detrusor overactivity and overactive bladder. Brain control of normal and overactive bladder has been examined in humans using functional brain imaging (9, 11). Further work in animal experiments coupled with clinical studies in humans should aim to examine how the regions in the PAG and other parts of the brain interact to achieve normal urinary continence.

In conclusion, this study is the first report revealing the existence of micturition-suppressing region at the dorsolateral margin of the rostral PAG that inhibits bladder contractions without increasing activity of the external urethral sphincter. Although GABA is assumed to be one of neurotransmitters that are involved in the PMC inhibition from the micturition-suppressing region in the PAG, further studies are warranted to clarify neurotransmitters and neural communications between the micturition-suppressing region in the PAG and the PMC. Despite these limitations, the present study will contribute to the understanding of the anatomy and physiology of the central nervous system that is involved in the control of micturition.

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**REFERENCES**


