Effect of (±)-epibatidine, a nicotinic agonist, on the central pathways controlling voiding function in the rat

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Lee, Sun-Ju, Yasuo Nakamura, and William C. de Groat. Effect of (±)-epibatidine, a nicotinic agonist, on the central pathways controlling voiding function in the rat. Am J Physiol Regul Integr Comp Physiol 285: R84–R90, 2003.—Nicotinic receptors in the brain modulate the release of many transmitters that are known to regulate voiding. This prompted us to examine the central nervous system effects of a neuronal nicotinic agonist, (±)-epibatidine, on voiding function in awake and anesthetized rats. Intracerebroventricular injection of (±)-epibatidine (0.1 μg) significantly increased intercontraction interval (ICI) but did not change pressure threshold (PT) or maximal voiding pressure (MVP), whereas 1 μg of (±)-epibatidine increased PT and MVP (P < 0.05) and decreased ICI. A low intravenous dose of (±)-epibatidine (0.001–0.1 μg) had no effect; however, a large dose of (±)-epibatidine (1 μg) significantly decreased ICI and increased MVP (P < 0.05) but did not change PT (P > 0.05). The effects occurred within 5–10 min after injection and persisted for 1–2 h. Intracerebroventricular chlorisondamine (10 μg), a nicotinic receptor antagonist, blocked the effect of intracerebroventricular (±)-epibatidine (0.1 μg). The experiments revealed that activation of nicotinic receptors in the brain increased bladder capacity in awake and anesthetized rats. These results suggest that the nicotinic agonist can activate mechanisms that inhibit voiding reflexes.

nicotinic acetylcholine receptors; central nervous system; chlorisondamine

CHOLINERGIC MECHANISMS are involved at various sites in the neural pathways controlling voiding function. In the peripheral nervous system acetylcholine is the major excitatory transmitter 1 at the parasympathetic neuroeffector junction in the bladder, 2 in sympathetic and parasympathetic ganglia, and 3 at the neuromuscular junction in urethral striated muscle (6, 12). In the bladder the effects of acetylcholine are mediated by muscarinic receptors, whereas in ganglia and at the neuromuscular junction nicotinic receptors are involved (5).

Experimental studies in animals have raised the possibility that acetylcholine is also a transmitter in the central neural pathways controlling bladder activity. Injections of cholinergic agonists into the region of the pontine micturition center in cats elicited inhibition or excitation, depending on the site of the injection (21). The effects were antagonized by atropine, indicating a role of muscarinic receptors. Administration of bethanechol, a muscarinic agonist, to the brain of the dog by a vascular cross-perfusion technique reduced bladder capacity and increased maximal voiding pressure (MVP) (14). In the rat, intracerebroventricular injection of oxotremorine-M, a muscarinic agonist, elicited both inhibitory and excitatory effects that were blocked by atropine (9). Intracerebroventricular administration of atropine alone decreased MVP and increased bladder capacity, indicating that excitatory mechanisms are tonically active in controlling voiding function. Recent experiments revealed that second messenger pathways involving protein kinase C are involved in the central muscarinic inhibitory control of bladder activity in the rat (13).

The demonstration of central muscarinic cholinergic mechanisms in micturition raises the possibility that nicotinic receptors might also be involved in the control of voiding function. Nicotinic receptors are widely distributed in the central nervous system (CNS; 20) and have a prominent modulatory effect on various types of synaptic transmission. Activation of nicotinic receptors on presynaptic terminals enhances the release of many transmitters, including norepinephrine, dopamine, glutamic acid, acetylcholine, and serotonin (5-HT) (3, 10, 17, 18, 24), all of which have been implicated in the central nervous control of bladder function (5).

Administration of nonselective neuronal nicotinic receptor agonists such as nicotine or (±)-epibatidine [exo-2-{6-chloro-3-pyridyl}-7-azabicyclo[2.2.1], a toxic agent from frog skin] produces prominent changes in CNS activity, including improvement in cognitive functions, changes in motor behavior, analgesia, nausea, and vomiting (4, 11, 22, 25). Agents with selective nicotinic receptor agonist actions have recently been synthesized (4) and are being evaluated for their efficacy in the treatment of various disorders ranging from Alzheimer’s disease, pain, to attention deficit hyperactivity disorder (1).
In the present experiments we examined the role of central nicotinic receptors in micturition by studying the effect of intracerebroventricular and intravenous administration of the nicotinic agonist \((\pm\)-epibatidine and the nicotinic antagonist chlorisondamine on voluntary and reflex voiding function in awake and anesthetized female rats, respectively.

**MATERIALS AND METHODS**

**Animal preparations.** Voiding was studied in urethane-anesthetized (1.2 g/kg sc) or awake female Sprague-Dawley rats (250–300 g, Hilltop Lab, Scottsdale, PA). In the latter group of animals, surgery was performed under halothane anesthesia (2% in oxygen). To implant the intracerebroventricular catheter, the rats were positioned in a stereotaxic frame, a scalp incision was made over the sagittal suture, and a hole (diameter ~1.0 mm) was drilled in the right parietal bone to expose the dural surface 1.0 mm lateral and 0.3 mm anterior to the bregma according to the atlas coordinates of Paxinos and Watson (15). A sterile, stainless steel catheter (OD 0.6 mm, ID 0.3 mm, length 10.5 mm) was lowered 5.3 mm ventrally from the bregma with a micromanipulator. With the aid of a small screw placed in the skull as an anchor, the catheter was fixed to the skull with dental acrylic. Solutions were injected via an infusion catheter (OD 0.3 mm, ID 0.1 mm) inserted into the larger catheter. Single doses of drugs were administered in a volume of 1 \(\mu l\) over a period of 1 min, and the infusion catheter was left in place for 1 min after injection to allow for diffusion of the drug solution. At the end of the experiment, Blue Food Color (McCormick, Hunt Valley, MD) was injected to verify the location of the cannula tip. For intravenous administration of drugs, a catheter (PE-50) was placed in the left jugular vein. Single volumes of drug solutions were administered intravenously in sterile physiological saline (0.9% sodium chloride solution) followed by a 200-\(\mu l\) flush of saline.

The urinary bladder was catheterized using the method of Yaksh et al. (26). The bladder was exposed via a midline abdominal incision. A catheter (PE-50), the bladder end of which was heated to create a collar, was inserted through a small incision in the bladder dome, and a suture was tightened around the collar. The other end of the catheter was passed through subcutaneous tissue and exited through the skin. After closing the abdominal incision by suturing the muscle and skin, rats that were to be studied without anesthesia were administered anesthetics or saline containing a nicotinic acetylcholine receptor (nAChR) antagonist, was injected into the bladder at a constant rate of 0.04 \(\text{cmH}_2\text{O} \cdot \text{sec}^{-1}\) and a small, but insignificant rise in bladder pressure occurred 2 min after \((\pm\)-epibatidine injection, was also noted. These parameters were measured for each CMG.

**Drugs.** Drugs used in this study included halothane (Ayerst Lab, Philadelphia, PA), urethane (Sigma Chemical, St. Louis, MO), \((\pm\)-epibatidine [neuronal nAChR agonist; Sigma Chemical], chlorisondamine (nAChR antagonist; Ciba Geigy, Summit, NJ). For intracerebroventricular injection, \((\pm\)-epibatidine and chlorisondamine were dissolved in aCSF (8).

**Evaluation.** In all experiments, control cystometrograms (CMGs) were recorded for 2 h before intracerebroventricular and intravenous injection of vehicle or drug solutions. Dose-response curves were constructed by administering increasing doses of \((\pm\)-epibatidine [0.001–1 \(\mu g\) in 1 \(\mu l\) intracerebroventricularly (icv); 0.001–1 \(\mu g\) in 200 \(\mu l\) iv] at 30-min to 2-h intervals. \((\pm\)-Epibatidine was administered ~30 min after vehicle \([\text{artificial cerebrospinal fluid (aCSF), 1 \(\mu l\) iv; or saline solution, 200 \(\mu l\) iv}]). Chlorisondamine (10 \(\mu g\), 1 \(\mu l\) iv) was a nicotinic acetylcholine receptor (nAChR) antagonist, was injected 10–30 min before \((\pm\)-epibatidine via the intracerebroventricular route in some experiments to block the effect of the agonist. The intravesical pressure to induce micturition \([\text{pressure threshold (PT)}, MVP, \text{and intercontraction interval (ICI); the interval between voids or reflex bladder contractions}) were measured (28) and converted into percent change from control values. The presence of nonvoiding contractions, occurring at peak pressures >10 \(\text{cmH}_2\text{O}\) during bladder filling after \((\pm\)-epibatidine injection, was also noted. These parameters were measured for each CMG.

**RESULTS**

**Effect of \((\pm\)-epibatidine via intracerebroventricular injection in awake rats.** In awake rats \((n = 7)\) in which voiding parameters were not changed after intracerebroventricular injection of aCSF (ICI: 521.8 ± 158.4 s, PT: 10.2 ± 3.0 \(\text{cmH}_2\text{O}\), MVP: 35.1 ± 1.5 \(\text{cm H}_2\text{O}\)), \((\pm\)-epibatidine elicited changes in the cystometrogram that varied according to the dose (Fig. 1). Low doses of \((\pm\)-epibatidine \((0.001–0.01 \mu g)\) did not alter any CMG parameter, whereas a moderate dose \((0.1 \mu g)\) significantly increased the ICI \((217 ± 41.4\% \text{of control})\) \((P < 0.05)\) but did not change PT \((140.0 ± 9.8\% \text{of control})\) or MVP \((112.5 ± 10.1\% \text{of control})\). The change in the ICI was apparent within 5–10 min. A high dose \((1 \mu g)\) of \((\pm\)-epibatidine induced a large increase in bladder pressure \(\text{(see Figs. 2 and 3)\) accompanied by movement of the animal and suppression of respiration. The rise in bladder pressure occurred 2–3 min after injection and persisted for 10 s–3 min and was followed by recovery of intermittent voiding, which occurred at significantly \((P < 0.05)\) increased PT \((167.1 ± 6.3\% \text{of control})\) and MVP \((173.1 ± 13.1\% \text{of control})\) \((P < 0.05)\) and a small, but insignificant, decrease in the ICI \((64.3 ± 7.0\% \text{of control})\) \((P > 0.05)\). Nonvoiding contractions occurred after the high dose \((1 \mu g)\) of \((\pm\)-epibatidine but not after lower doses. Duration of action was 1–2 h (Fig. 2).

Intracerebroventricular injection of chlorisondamine \((10 \mu g \text{in } 1 \mu l)\) in awake rats did not change CMG parameters significantly. However, this dose of chlorisondamine administered 10–30 min before \((\pm\)-epibatidine blocked the effects of a moderate dose \((0.1 \mu g)\) of \((\pm\)-epibatidine but did not block the initial large rise in intravesical pressure, the movements or sup-
pression of respiration induced by 1 μg (±)-epibatidine (Fig. 3). In chlorisondamine-pretreated rats, (±)-epibatidine (1 μg) did not change the ICI (98.8 ± 30.1% of control) (P > 0.05).

Effect of (±)-epibatidine via intracerebroventricular injection in urethane-anesthetized rats. In anesthetized rats (n = 4), voiding parameters were not changed after injection of aCSF (ICI: 251.6 ± 74.9 s, PT: 13.8 ± 1.5 cmH2O, MVP: 28.2 ± 1.5 cmH2O). Low intracerebroventricular doses of (±)-epibatidine (0.001–0.01 μg) did not significantly change (P > 0.05) the CMG. However, 0.1 μg (±)-epibatidine significantly increased the ICI (191.3 ± 26.9% of control) without significantly changing PT (128.5 ± 15.9% of control) or MVP (122.6 ± 4.8% of control). A high dose (1 μg) of (±)-epibatidine also induced an initial large increase in bladder pressure and suppression of respiration similar to the effects elicited in awake rats. The high dose of (±)-epibatidine (1 μg) also significantly increased the MVP (204.1 ± 12.1% of control) and PT (182.3 ± 42.7% of control) (P < 0.05) but did not significantly (P > 0.05) change the ICI (78.8 ± 33.2% of control) (Fig. 4).

Fig. 1. Effects of a moderate and high dose of intracerebroventricular (±)-epibatidine on the cystometrograms (CMGs) in the awake rat. CMGs were recorded 0 to 30 min after intracerebroventricular injection of (±)-epibatidine. Note artificial cerebrospinal fluid (aCSF) and low dose of (±)-epibatidine did not affect the intercontraction interval (ICI). A moderate dose of (±)-epibatidine increased the ICI, whereas a high dose of (±)-epibatidine (bottom trace) induced an initial bladder contraction and decreased the ICI.

Fig. 2. Duration and reproducibility of the effect of intracerebroventricular (±)-epibatidine in the awake rat. aCSF did not affect CMG parameters (top trace). Moderate dose (0.1 μg) of (±)-epibatidine increased ICI, which gradually recovered over the course of 90 min. Second administration (0.1 μg) of (±)-epibatidine produced a similar effect (bottom trace).
Effect of (±)-epibatidine via intravenous injection in urethane-anesthetized rats and awake rats. Intravenous injection of normal saline did not alter any CMG parameter in anesthetized (n = 4) or awake rats (n = 6) (ICl: 257.3 ± 93.7 and 207 ± 60 s, PT: 11.0 ± 2.9 and 14.1 ± 1.8 cmH₂O, MVP: 32.4 ± 4.0 and 34.6 ± 7.9 cmH₂O). Low and moderate intravenous doses of (±)-epibatidine (0.001–0.1 µg) did not significantly change

![Graphs showing changes in CMG parameters](image_url)

Fig. 3. Inhibitory effects of intracerebroventricular (±)-epibatidine (0.1 µg) on voiding were blocked by pretreatment with intracerebroventricular chlorisondamine in the awake rat (3rd trace). However, the initial excitatory effect of 1 µg (±)-epibatidine was not antagonized. CMGs were recorded 0 to 30 min after intracerebroventricular injection of each solution. Tracings represent continuous recordings of bladder pressure.

![Graphs showing changes in CMG parameters](image_url)

Fig. 4. Changes in CMG parameters after intracerebroventricular injection of (±)-epibatidine in awake (n = 7) and anesthetized (n = 4) rats. Each histogram represents mean ± SD. PT, pressure threshold; MVP, maximal voiding pressure. *P < 0.05 compared with the effect of aCSF (% of control).
ICI, PT, and MVP in anesthetized and awake rats. However, a high dose (1 µg) of (±)-epibatidine significantly decreased ICI (anesthetized 33.6 ± 8.5% of control, awake 45.2 ± 7.1% of control) and increased MVP (anesthetized 190.5 ± 40.2% of control, awake 185.5 ± 10.0% of control) but did not significantly change PT (anesthetized 122.3 ± 50.6% of control, awake 161.6 ± 14.3% of control) (Fig. 5).

**DISCUSSION**

The present results indicate that activation of nicotinic receptors in the brain by small doses of (±)-epibatidine increases the voiding interval during continuous infusion cystometry in both awake and anesthetized rats without altering voiding pressure. This effect in awake rats was blocked by intracerebroventricular injection of chlorisondamine, a neuronal nAChR antagonist, in doses that alone did not alter voiding function. These findings suggest that nicotinic mechanisms in the brain have the potential for exerting an inhibitory influence on micturition but that under the conditions of our experiments these mechanisms were inactive before administration of (±)-epibatidine.

Because (±)-epibatidine is able to pass through the blood-brain barrier, one question that arises in the present experiments is the site of action of the drug after intracerebroventricular administration. Nicotinic receptors are not only present in the CNS but also in peripheral autonomic ganglia in the sympathetic and parasympathetic efferent pathways to the urinary bladder, and therefore it is possible that (±)-epibatidine administered intracerebroventricularly could diffuse into the systemic circulation and alter voiding function by an action in the peripheral nervous system. However, this seems unlikely for several reasons. First, moderate doses of (±)-epibatidine (0.1 µg) that were active by intracerebroventricular injection were inactive after intravenous injection. Second, the effect of (±)-epibatidine after intracerebroventricular injection occurred within 5–10 min. This time of onset is similar to that of other agents administered intracerebroventricularly or systemically (9, 16) and seems insufficient time for a direct action on the peripheral nervous system. Third, chlorisondamine, a quaternary drug that is retained in the brain after intracerebroventricular injection, blocked the effect of 0.1 µg (±)-epibatidine. This antagonism should only occur if (±)-epibatidine were acting in the brain.

However, the site of action of high doses of (±)-epibatidine, which produced a rise in intravesical pressure and reduced the voiding interval, is less clear because large doses of (±)-epibatidine administered intravenously produced similar effects and chlorisondamine injected intracerebroventricularly was not able to block the effect of (±)-epibatidine. Thus large intracerebroventricular doses of (±)-epibatidine might enter the peripheral circulation and stimulate parasympathetic ganglia to induce a bladder contraction. Alternatively, large doses of (±)-epibatidine might act in the CNS on nAChRs that had not been accessed by chlorisondamine due to the relatively short pretreatment
time. Chlorisondamine is more hydrophilic than (±)-epibatidine and therefore is likely to require a long time for diffusion throughout the brain. Another concern in interpreting the effect of the large dose of (±)-epibatidine is that this dose induced respiratory changes as well as limb and body movements. Thus it is possible that changes in intra-abdominal pressure were transmitted to the bladder, and this contributed to the apparent facilitatory effect of the drug on voiding.

(±)-Epibatidine has not only a potent stimulatory effect on nAChR subtypes in the brain (α4β2) but also in sympathetic and parasympathetic ganglia (α3β4) and at the neuromuscular junction (α1β1γδ) (22). Thus the excitatory effects of intravenous injection of (±)-epibatidine (1 μg) on voiding function shown by decreased ICI and increased MVP could be due to stimulation of the parasympathetic excitatory input to the bladder as well as stimulation of sympathetic input to the urethral smooth muscle or direct stimulation of nicotinic receptors in the striated muscle of the urethra, which would increase outlet resistance. Effects of (±)-epibatidine on peripheral nAChR can also produce hypertension and striated muscle effects, both of which limit the clinical use of nicotinic agonists (19, 22).

Although the CNS site of the inhibitory action of (±)-epibatidine on micturition is uncertain, it seems reasonable to conclude that the drug depresses the afferent limb of the micturition reflex in anesthetized rats and depresses the sensory pathways that induce voluntary voiding in awake rats because the drug decreased voiding frequency without altering voiding pressure. If the efferent pathway from the brain to the sacral parasympathetic nucleus or sphincter motor nucleus in the spinal cord were affected by the drug, it would be expected that peak voiding pressure would be changed. The decrease in voiding frequency after (±)-epibatidine could be produced by inhibition of transmission of afferent signals in the spinal cord or inhibition of afferent processing in the brain stem pontine micturition center or in centers in the forebrain involved in voluntary voiding (Fig. 6). However, there was no significant difference between the effect of (±)-epibatidine on CMG parameters in urethane-anesthetized and awake rats. Because voiding in anesthetized animals is reflex and presumably involves circuitry in the brain stem and spinal cord but is not dependent on forebrain circuits, this raises the possibility that (±)-epibatidine acted on the brain stem to alter voiding in both groups of animals.

Activation of nAChRs is known to enhance the release of various CNS transmitters, including glutamate, 5-HT, norepinephrine, acetylcholine, and dopamine (3, 10, 17, 18, 24). Previous pharmacological studies in animals showed that activation of the receptors for these transmitters in the brain can under certain conditions inhibit voiding function, reduce voiding frequency, and increase bladder capacity (7, 23, 27). For example, activation of descending 5-HT pathways projecting from the brain stem raphe nucleus to the spinal cord can increase bladder capacity and produce effects similar to those induced by (±)-epibatidine. Activation of bulbospinal 5-HT pathways has also been implicated in the analgesic effects of neuronal nicotinic agonists (2). In addition, activation of muscarinic receptors in the brain or spinal cord can increase the ICI, raising the possibility that an enhancement of ACh release by (±)-epibatidine can be involved in its inhibitory effect on voiding. Thus it will be important to evaluate the downstream mechanisms involved in the suppression of voiding by nicotinic receptor agonists.

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REFERENCES


