Glutamatergic and dopaminergic contributions to rat bladder hyperactivity after cerebral artery occlusion

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Yokoyama, Osamu, Mitsuharu Yoshiyama, Mikio Nakiki, and William C. De Groat. Glutamatergic and dopaminergic contributions to rat bladder hyperactivity after cerebral artery occlusion. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R935–R942, 1999.—The contribution of glutamatergic and dopaminergic mechanisms to bladder hyperactivity after left middle cerebral artery occlusion was evaluated by determining the effects of intravenous cumulative doses of an N-methyl-D-aspartate (NMDA) glutamatergic antagonist (MK-801) and D1-selective (Sch-23390), D2-selective (sulpiride), or nonselective (haloperidol) dopaminergic antagonists on bladder activity in sham-operated (SO) and cerebral-infarcted (CI) rats. MK-801 (1 and 10 mg/kg) or sulpiride (3–30 mg/kg) significantly increased bladder capacity (BC) in CI but decreased or had no effect, respectively, on BC in SO. Sch-23390 (0.1–3 mg/kg) decreased BC in both SO and CI. In both CI and SO, low doses of haloperidol (0.1–1 mg/kg) increased BC, but a higher dose (3 mg/kg) reversed this effect. Administration of haloperidol (0.3 mg/kg) or sulpiride (10 mg/kg) in combination with MK-801 (0.01–10 mg/kg) markedly increased BC in CI but produced small decreases or increases in BC depending on the dose of MK-801 in SO. These results indicate that the bladder hyperactivity induced by cerebral infarction is mediated in part by NMDA glutamatergic and D2 dopaminergic excitatory mechanisms.

Glutamate, a major excitatory neurotransmitter in the central nervous system, enhances bladder activity, i.e., an increase in bladder contraction amplitude or a decrease in bladder capacity, after injection into the PMC and other sites in the brain stem of the cat and rat (2, 8, 10, 19). MK-801 (dizocilpine), a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, depresses bladder and external urethral sphincter (EUS) activity in urethan-anesthetized animals (9, 25, 27), whereas in unanesthetized rats, MK-801 facilitates bladder activity but still depresses EUS activity (17, 21, 22). These observations suggest that a glutamatergic pathway using NMDA receptors plays a role in both excitatory and inhibitory regulation of micturition.

There is also evidence to indicate that D2-like dopaminergic receptors (hereafter called D2), which include D2, D3, and D4, functionally couple to a G protein and may modulate ion channels (24). MK-801 (0.01–10 mg/kg) or sulpiride (3–30 mg/kg) significantly increased bladder capacity (BC) in CI but decreased or had no effect, respectively, on BC in SO. Sch-23390 (0.1–3 mg/kg) decreased BC in both SO and CI. In both CI and SO, low doses of haloperidol (0.1–1 mg/kg) increased BC, but a higher dose (3 mg/kg) reversed this effect. Administration of haloperidol (0.3 mg/kg) or sulpiride (10 mg/kg) in combination with MK-801 (0.01–10 mg/kg) markedly increased BC in CI but produced small decreases or increases in BC depending on the dose of MK-801 in SO. These results indicate that the bladder hyperactivity induced by cerebral infarction is mediated in part by NMDA glutamatergic and D2 dopaminergic excitatory mechanisms.
tion was markedly reduced, indicating a hyperactive bladder. Previous experiments indicated that glutamatergic pathways using NMDA receptors may play a role in hyperactivity (21, 22). However, an NMDA glutamatergic agonist cannot completely reverse the effect of cerebral infarction-induced bladder hyperactivity in awake animals (21), indicating that other transmitters may be involved. This study was undertaken to investigate the role of central NMDA and/or dopamine receptors in this type of bladder hyperactivity and interactions between these two receptor systems.

MATERIALS AND METHODS

All experiments were performed in strict accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Female Sprague-Dawley rats (n = 108) weighing 245–295 g (mean = 269 g) were used in this study.

Surgical procedure for implantation of cystometry catheter and MCA occlusion. The method of Yaksh and co-workers (20) was used to perform cystometry in awake rats. Animals were anesthetized with halothane (2%), and the bladder was exposed via a midline abdominal incision. The bladder end of a polyethylene catheter (PE-60; Clay-Adams, Parsippany, NJ) was heated to create a colar and passed through a small incision at the apex of the bladder dome, and a suture was tightened around the colar of the catheter. The catheter was tunneled subcutaneously and exited through the skin at the back of the animal.

After the abdominal skin was sutured, the left carotid bifurcation was exposed through a midline incision in the neck. After ligation of the left common carotid artery (ICA) was isolated and carefully separated from the adjacent vagus nerve. Then the pterygopalatine branch of the left ICA was ligated close to its origin. A 4–0 monofilament nylon thread whose tip had been rounded by exposure to a flame was inserted into the left ICA and advanced a distance of 17 mm from the carotid bifurcation to the origin of the left middle cerebral artery (MCA). This occluded blood flow in the left MCA and induced an infarction on the left side of the brain (7). In sham-operated animals, the left carotid bifurcation was exposed through a midline incision in the neck, but no further procedures were performed. After the jugular vein was cannulated (PE-10) for intravenous administration, rats were placed in a restraining cage and allowed to recover from halothane anesthesia.

Bladder activity (including bladder capacity, intravesical voiding pressure, and voided volume) was monitored via the cystometry catheter connected to a pressure transducer. Cystometric recordings were performed by infusing physiological saline at room temperature into the bladder at a rate of 0.04 ml/min. Saline voided from the urethral meatus was collected and measured to determine the voided volume. By evaporating the bladder through the cystometry catheter, we measured residual volume after the micturition reflex. Two cystometric parameters (bladder capacity and bladder contraction pressure) were determined from each cystometry. Bladder capacity was defined as the sum of the voided and residual volumes.

Effects of haloperidol, Sch-23390, sulpiride, and MK-801 on bladder activity. One hour after the left MCA occlusion or sham operation, the effects of increasing doses of haloperidol (a nonselective dopaminergic receptor antagonist, 0.01–3 mg/kg iv), Sch-23390 (a D1 dopaminergic receptor antagonist, 0.03–3 mg/kg iv), sulpiride (a D2 dopaminergic receptor antagonist, 0.1–30 mg/kg iv), MK-801 (0.01–10 mg/kg iv), or vehicle (0.9% saline) on bladder activity were examined in awake rats after control cystometric recording. Increasing doses of the drugs were administered cumulatively at 60-min intervals.

Interactions between MK-801 and haloperidol or between MK-801 and sulpiride. To examine the effects of multiple injections of a fixed dose of haloperidol or sulpiride on bladder capacity, repetitive administration at 1-h intervals of 0.3 mg/kg of haloperidol or 10 mg/kg of sulpiride was carried out in sham-operated rats and cerebral-infarcted rats. Seven injections were administered to the same animal.

Increasing doses of MK-801 (0.01–10 mg/kg iv) were administered to awake rats in combination with haloperidol (0.3 mg/kg iv) or sulpiride (10 mg/kg iv). MK-801 was injected simultaneously with haloperidol or sulpiride every hour.

Drugs. Drugs used in this study included MK-801 (dizocilpine; Merck Sharp & Dohme, West Point, PA), haloperidol (Research Biochemicals International, Natick, MA), Sch-23390 (Research Biochemicals International), and sulpiride (Research Biochemicals International). All drugs were dissolved in 0.9% saline for intravenous administration.

Data analysis. Data are expressed as mean values ± SE. Statistical comparisons were performed by one-way or two-way repeated-measures ANOVA, with subsequent individual comparisons by Fisher’s protected least significant difference test. A level of P < 0.05 was considered statistically significant.

RESULTS

Effects of haloperidol, Sch-23390, and sulpiride on bladder activity in sham-operated and cerebral-infarcted rats. Low doses of haloperidol (0.1–1 mg/kg iv) elicited small but significant increases in bladder capacity in both sham-operated and cerebral-infarcted rats compared with vehicle administration (Fig. 1A). The percentage increases in bladder capacity at 0.3 mg/kg of haloperidol in sham-operated and cerebral-infarcted rats were 30.1 ± 7.5 and 47.4 ± 8.0%, respectively. However, a larger dose (3 mg/kg) reversed the effects of low doses in both sham-operated and cerebral-infarcted rats. After the largest dose (3 mg/kg) of haloperidol, bladder capacity was not significantly different from that in the control period before the first dose of the drug. The effects of haloperidol were not significantly different in sham-operated and cerebral-infarcted rats. The amplitude of bladder contractions was not altered in sham-operated or cerebral-infarcted rats after administration of haloperidol (Fig. 1B).

Sch-23390 produced a significant decrease in bladder capacity (28.0–43.6%) in sham-operated rats in doses between 0.1 and 3 mg/kg, whereas in cerebral-infarcted rats, a significant decrease (30.0%) occurred only at 3 mg/kg (Fig. 2A). There was no significant difference in the percentage decrease at the largest dose in sham-operated or cerebral-infarcted rats.

Sulpiride significantly increased bladder capacity (39.4–60.0%) in cerebral-infarcted rats at doses between 3 and 30 mg/kg but did not elicit a significant change at any dose in sham-operated rats (Fig. 2B).

Neither Sch-23390 nor sulpiride altered the amplitude of bladder contractions in sham-operated or cerebral-infarcted rats (Table 1).
Effects of MK-801 on bladder activity in sham-operated and cerebral-infarcted rats. Low doses of MK-801 (0.01 and 0.1 mg/kg iv) reduced bladder capacity in sham-operated rats (Figs. 3A and 4A) but did not significantly change capacity in cerebral-infarcted rats (Figs. 3B and 4A). The percentage decrease in bladder capacity at 0.1 mg/kg of MK-801 in sham-operated rats was 60.7 ± 8.4%, versus 12.1 ± 8.6% in cerebral-infarcted rats (Fig. 4A, P < 0.01). In sham-operated rats, high doses of MK-801 (1 and 10 mg/kg iv) reversed...
the effect of low doses, but the capacity after 1 mg/kg of MK-801 was still significantly lower than control level (32.2 ± 14.4% of control capacity, *P*, 0.05). High doses of MK-801 (1 and 10 mg/kg iv) significantly increased bladder capacity in cerebral-infarcted rats (94.5 ± 18.7% of control capacity at 1 mg/kg, *P*, 0.05; 163.3 ± 24.9% at 10 mg/kg, *P*, 0.01). Residual volume expressed as a percentage of total bladder capacity (residual volume/bladder capacity × 100) after 10 mg/kg of MK-801 was not significantly changed in sham-operated (6.8%) or cerebral-infarcted rats (8.9%). No significant changes in the amplitude of bladder contractions were detected after any dose of MK-801 in sham-operated or cerebral-infarcted rats.

Interactions between MK-801 and haloperidol or between MK-801 and sulpiride in sham-operated and cerebral-infarcted rats. To study the interactions between MK-801 and the dopaminergic antagonists, we decided to administer multiple fixed doses of the dopaminergic antagonists while constructing a cumulative dose-response curve for MK-801. This treatment paradigm was necessary because haloperidol (0.3 mg/kg) and sulpiride (10 mg/kg) in the doses used are known to have a short duration of action (<1 h) (6, 24). These doses were selected because they produced significant increases in bladder capacity in cerebral-infarcted animals.

Single doses of haloperidol (0.3 mg/kg) produced an increase in bladder capacity, which reached maximum value within 20 min after administration and returned to control within 1 h (Fig. 5). Repeated administrations (7 times) yielded similar effects, with no change in baseline parameters. To the contrary, repetitive administration of a single dose of sulpiride (10 mg/kg) gradually increased baseline bladder capacity. For the first four administrations of sulpiride, bladder capacity returned close to baseline. However, after five injections, cumulative effects of sulpiride on bladder capacity appeared (Fig. 5).

Figure 3 shows the difference in the effect on bladder capacity between cumulative doses of MK-801 alone and combined administration of MK-801 and haloperidol or MK-801 and sulpiride. To compare the difference between sham-operated and cerebral-infarcted rats, we calculated percentage changes in bladder capacity from the data of Fig. 3 and present them in Fig. 4.

When increasing doses of MK-801 were administered to rats in combination with either 0.3 mg/kg haloperidol or 10 mg/kg sulpiride, marked differences in effects were noted between sham-operated and cerebral-
in cerebral-infarcted rats, these drug combinations (MK-801 and haloperidol and MK-801 and sulpiride) produced significant increases in bladder capacity even at low doses of MK-801 (P < 0.05 at 0.01 and 0.1 mg/kg; Fig. 3B). Furthermore, increases in bladder capacity produced by these drug combinations significantly differed from those produced by the administration of MK-801 alone (P < 0.01–0.05 at 0.01 and 0.1 mg/kg).

When the percentage changes in bladder capacity induced by drugs in sham-operated and cerebral-infarcted rats are compared, the differences are highly significant (Fig. 4, B and C). In sham-operated rats, combined administration of haloperidol and MK-801 produced a small decrease (6.4% at 0.01 mg/kg of MK-801, 28.4% at 0.1 mg/kg) or increase (39.8% at 10 mg/kg of MK-801) in bladder capacity; however, in cerebral-infarcted rats, bladder capacity markedly increased by 48.0–271.6% of control capacity (P < 0.01 at 0.01, 0.1, and 1 mg/kg of MK-801; Fig. 4B). Percentage residual volume at 10 mg/kg of MK-801 in sham-operated rats was 20.5%, versus 36.9% in cerebral-infarcted rats (not significantly different). No significant changes in the amplitude of bladder contractions were detected with this drug combination.

In combination with 10 mg/kg of sulpiride, MK-801 produced significantly different effects on bladder capacity in sham-operated and cerebral-infarcted rats (Fig. 4C). At low doses of MK-801 (0.01 and 0.1 mg/kg), this combination decreased bladder capacity in sham-
operated rats by 20.7 and 35.6%, respectively. On the contrary, this combination significantly increased bladder capacity in cerebral-infarcted rats at doses between 0.01 and 10 mg/kg of MK-801 (36.1 - 251% of control capacity; P < 0.01). Percentage residual volume after 10 mg/kg MK-801 in sham-operated rats was 29.6%, versus 16.4% in cerebral-infarcted rats (not significantly different). No significant changes in the amplitude of bladder contractions were detected with this drug combination.

DISCUSSION

The present study evaluated the contribution of NMDA glutamatergic and dopaminergic mechanisms to bladder hyperactivity in cerebral-infarcted awake rats. As noted in recent experiments (21, 22), cerebral infarction markedly reduced bladder capacity. Administration of 1 and 10 mg/kg of MK-801 reversed this effect of cerebral infarction. On the contrary, administration of MK-801 to sham-operated rats produced the opposite effect, i.e., a reduction in bladder capacity. These results suggest that NMDA glutamatergic mechanisms have an essential role in the bladder hyperactivity induced by cerebral infarction. High doses of sulpiride also increased bladder capacity in cerebral-infarcted rats, an effect not seen in sham-operated animals. These data indicate that D2 dopaminergic excitatory mechanisms also contribute to the bladder hyperactivity after cerebral infarction. This conclusion is further supported by the finding that MK-801 and either sulpiride or haloperidol administered in combination have additive effects to increase bladder capacity in cerebral-infarcted rats.

After systemic administration, MK-801 and dopaminergic antagonists could act at various sites in the central and peripheral nervous system to influence voiding function. However, the finding that MK-801 or sulpiride act only in cerebral-infarcted but not normal rats to selectively increase bladder capacity without altering intravesical voiding pressure eliminates certain potential sites of action and allows the formulation of a hypothetical schema for explaining the effects of these drugs. For example, an action on the peripheral afferent or efferent autonomic pathways seems unlikely because an action at these sites would have altered bladder activity in sham-operated control animals. In addition, a depressant effect on efferent pathways would have decreased the amplitude of bladder contractions. However, this did not occur. Thus an influence of the drugs on the PMC seems more likely because this part of the micturition reflex circuitry is known to strongly regulate bladder capacity. The drugs could act directly on neurons or synapses in the PMC or act at other sites in the brain that project to the PMC.

In this regard, it is important to note that there are at least two glutamatergic systems in the brain that control the micturition reflex pathway. One glutamatergic system that has a low sensitivity to MK-801 originates in the brain stem and mediates an excitatory control of voiding (21). The other system, which is more sensitive to MK-801, originates in the forebrain and activates an inhibitory mechanism that controls bladder capacity. Thus, in awake normal rats (17, 21) or the awake sham-operated rats in the present study, a low dose of MK-801 increased the frequency of bladder contractions and decreased bladder capacity by blocking the inhibitory system. This did not occur in cerebral-infarcted rats. On the other hand, in cerebral-infarcted rats, a higher dose of MK-801 increased bladder capacity.

What accounts for this reversal of the effects of MK-801 after cerebral infarction? This may be mediated by two changes. First, the loss of the facilitatory effect of low doses is most reasonably attributed to elimination by cerebral infarction of the putative glutamate-dependent inhibitory pathway, indicating that the inhibition depends on tonic drive from the area of brain destroyed by the infarction. On the other hand, the effect of a higher dose of MK-801 to reverse the cerebral infarction-induced decrease in bladder capacity suggests that the brain stem glutamatergic excitatory pathway is upregulated by cerebral infarction. Thus cerebral infarction seems to alter two NMDA glutamatergic pathways, i.e., downregulation of a tonic inhibitory pathway and upregulation of an excitatory pathway.

The present experiments indicate that dopaminergic control of micturition is also altered by cerebral infarction. Dopamine is contained in high concentrations in the striatum, where it is found in nerve terminals originating from cell bodies in the substantia nigra. Occlusion of the left MCA produces ischemic infarcts in the ipsilateral frontoparietal cortex and in the caudate putamen (11). Because dopaminergic nerve terminals are very sensitive to ischemic damage (18), it is likely that there would be changes in dopaminergic systems controlling the micturition reflex. Dopaminergic pathways from the substantia nigra are reported to have an inhibitory effect on the micturition reflex via activation of D1 receptors, whereas other dopaminergic systems terminating in the brain stem activate facilitatory D2 receptors, resulting in a reduction in bladder capacity (3, 23, 24). In the present study, Sch-23390, a selective D1 receptor antagonist, facilitated the micturition reflex in both awake sham-operated and cerebral-infarcted rats presumably by blocking a tonic D1 inhibition.

On the other hand, sulpiride, a selective D2 receptor antagonist, produced a significant increase in bladder capacity in cerebral-infarcted rats but not in sham-operated rats. Because high doses of sulpiride (100 mg/kg ip) are required to suppress normal bladder activity (6), it is tempting to speculate that cerebral infarction initiates a resetting of the trigger point for micturition by upregulating D2 dopaminergic excitatory mechanisms in addition to changing glutamatergic pathways.

The effects of haloperidol are more complicated because it blocks both types of dopaminergic receptors. Low doses of haloperidol increased bladder capacity in cerebral-infarcted as well as sham-operated rats. It seems unlikely that in sham-operated animals this is
due purely to block of \( D_2 \) receptors, because sulpiride did not produce the same effect. A depressant effect of haloperidol on bladder activity in normal animals has also been reported by other investigators (6). These results raise the possibility that in normal animals haloperidol might alter other neurotransmitter systems controlling the micturition reflex, i.e., adrenergic or serotonergic mechanisms. On the other hand, in cerebral-infarcted animals, haloperidol and sulpiride elicited similar effects, presumably due to block of \( D_2 \) receptors. Likewise, a large dose of haloperidol, which reversed the effects of lower doses, might also act at other neurotransmitter systems.

Concerning the dopaminergic control of micturition reflex, it has been reported that the depressant effect of MK-801 on bladder contraction amplitude is antagonized by L-DOPA or apomorphine pretreatment in urethan-anesthetized rats (26), suggesting that dopaminergic receptor activity can enhance NMDA glutamatergic transmission and overcome the MK-801 blockade or that dopaminergic receptor excitation can substitute for the loss of the NMDA excitatory pathway. This is consistent with the proposal that glutamatergic and dopaminergic excitatory controls of micturition represent two parallel pathways, both of which appear to be upregulated by cerebral infarction. The effect of cerebral infarction to alter the interaction between glutamatergic and dopaminergic antagonists provides support for this hypothesis. In sham-operated animals, a low dose of MK-801 in combination with haloperidol or sulpiride decreased bladder capacity (i.e., facilitated micturition), whereas these combinations in cerebral-infarcted animals normalized bladder capacity. Thus when these two agents are combined, there appears to be an additive effect on bladder hyperactivity in cerebral-infarcted rats. This interaction will be evaluated in more detail in future experiments by examining the effects of more selective dopaminergic and glutamatergic antagonists on voiding function after cerebral infarction.

In other neural systems, additive effects of glutamatergic and dopaminergic drugs have also been reported; however, these interactions were opposite to those occurring in cerebral-infarcted rats. For example, a synergistic effect with regard to motor activity was observed when MK-801 was combined with a low (subthreshold) dose of the dopaminergic agonist apomorphine or with the selective \( D_1 \) receptor agonist SKF-38393 (1). Indeed, it has been suggested that combination therapies with these agents might be used to achieve an optimal beneficial effect with minimal side effects for certain neurological disorders (1).

In conclusion, multiple glutamatergic and dopaminergic mechanisms in the brain appear to exert excitatory or inhibitory influences on the micturition reflex. NMDA glutamatergic pathways seem to play a major role in supraspinal circuits regulating bladder capacity. The data suggest that cerebral infarction reduces bladder capacity by downregulating an NMDA-dependent inhibitory mechanism and upregulating an NMDA facilitatory mechanism. However, dopaminergic pathways, including \( D_2 \) excitatory and \( D_1 \) inhibitory types, also have a role and seem to interact with glutamatergic pathways. Because the two neurotransmitter systems seem to interact, combined drug therapies might be more effective than single drugs to treat hyperactivity of the bladder after cerebral infarction.

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Received 18 May 1998; accepted in final form 9 December 1998.

REFERENCES


