A Mechanistic Study on Urine Retention in \textit{D}-Amphetamine Addicts

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Abstract

Chronic amphetamine intake leads to neurogenic bladder and chronic urinary retention. The mechanism underlying persistent urinary retention is unclear. The pelvic-urethral reflex (PUR) is essential for the urethra to develop sufficient resistance to maintain urine continence, an important function of the urinary system. Recent studies on PUR activities have indicated that repetitive/tetanic stimulation of the pelvic afferent fibers induces spinal reflex potentiation (SRP) in PUR activities, which further increases urinary retention. In this study, results showed that test stimulation (TS, 1/30 Hz) evoked a baseline reflex activity, while repetitive stimulation (RS, 1 Hz) induced reflex potentiation in the external urethral sphincter. Intrathecal \textit{d}-amphetamine (AMPH, 30 \textmu M) did not but higher AMPH concentration (100 \textmu M) induced SRP in TS-induced reflex activity. H89 (10 \textmu M, a protein kinase A inhibitor), but not chelerythrine chloride (CTC, 10 \textmu M, a protein kinase C inhibitor), prevented the 100 \textmu M AMPH-elicited SRP. At 30 \textmu M, forskolin, an activator of adenylyl cyclase, elicited SRP. The co-administration of 10 \textmu M forskolin and 30 \textmu M AMPH induced SRP in TS-induced reflex activity. These results implied that the repetitive/tetanic stimulation of the pelvic afferent fibers could induce SRP in PUR activities, so that the urethra can produce sufficient resistance and played a significant role in urinary retention. Findings in this study demonstrated that amphetamine could induce bladder dysfunction by triggering protein kinase A activation, and provide a practical basis for the development of treatment for amphetamine-associated urinary retention.

Key Words: \textit{d}-amphetamine, micturition, pelvic afferent nerve, pelvic-urethra reflex, reflex plasticity, spinal reflex potentiation

Introduction

Amphetamines are a class of central nervous system (CNS) stimulants and have been used to treat narcolepsy, attention-deficit disorder and obesity (1, 24). Amphetamines induce numerous neurological effects such as feelings of euphoria, heightened self-confidence, sustained high energy levels and decreased
appetite (16, 22, 25). As powerful CNS stimulants, the use of amphetamines is widely abused (6, 11, 12, 16, 18, 22, 23). The most important amphetamines are amphetamine and (±)3,4-methylenedioxymethylamphetamine (MDMA), both of which are widely abused psychotropic drugs (16).

Chronic usage of amphetamine or MDMA has been reported to lead to neurogenic bladder and chronic urinary retention problems (2, 9). Of particular interest is the persistence of urinary retention despite the patients’ reported cessation of MDMA ingestion more than a month prior to presentation, supported by a negative urine drug screen, and continued participation in a drug-treatment program. The mechanism of amphetamine or MDMA abuse leading to persistent urinary retention is not clear.

Urine storage is one of the main functions of the urinary bladder. As the bladder is filled, mechanoreceptors on the bladder wall are excited and generate action potentials. These impulses then transmit centripetally into the dorsal horn neurons through the pelvic afferent nerve (PAN). After integration within the spinal cord the impulses excite the external urethral sphincter (EUS) and result in its contraction via the pudendal efferent nerve (PEN). This pelvic-pudendal reflex (PPR), sometimes called holding reflex, is essential for urine continence in the rat (3, 4, 8). Recent studies on pelvic-urethra reflex (PUR) activities have shown that repetitive/tetanic stimulation of the pelvic afferent fibers induces a distinct and long-lasting potentiation (LTP) in PUR activities. This LTP activity has been considered the mechanism underlying urine retention. The efficiency of reflex activity in the CNS is not constant and can be modulated by neuronal circuitry and synaptic transmission (21). For example, in hippocampus, repetitive activation of synaptic connections leads to enhancement of neural activity, which is now well-known as long term potentiation (LTP) (7, 14, 15, 19, 27). Studies investigating LTP have suggested that activation of the glutamatergic NMDA and AMPA receptors mediates long-lasting excitation of synapses (7, 15, 19, 25, 27). However, whether administration of amphetamine could modify the NMDA-associated neurotransmission to affect the bladder functions has not yet been clearly defined. This research aimed to characterize the effects of intrathecal administration of d-amphetamine (AMPH) on the PUR activity that contributes to the urine continence by the bladder.

**Materials and Methods**

**Animal Preparations**

Four hundred fifty-five female Wistar rats (200-250 g) were used in this experiment. They were anesthetized with intraperitoneal injection of urethane (1.2 g/kg). Study protocols were reviewed and approved by the Institutional Review Board at China Medical University (Taichung, Taiwan, ROC). All experimental procedures have been fully reported in previous studies (5). In brief, a PE-50 catheter (Portex, Hythe, Kent, UK) was placed in the left femoral vein for administration of anesthetics when needed. Body temperature was kept at 36.5 to 37.0°C by an infrared light and was monitored using a rectal thermometer. A midline abdominal incision was made to expose the pelvic viscera. Both ureters were ligated distally and cut. The left pelvic nerve was dissected carefully from the surrounding tissues and was transected as distally as possible for stimulation. The rats were monitored for corneal reflex and a response to noxious stimulation to the paw throughout the experiment. If either was present, a supplementary dose (0.4 g/kg) of anesthetics was given through the venous catheter. At the end of the experiment, the animals were sacrificed by an intravenous injection of potassium chloride saturation solution under deep anesthesia.

**Application of Drugs**

All drugs were dissolved in artificial cerebrospinal fluid for i.t. injection and these solutions were adjusted to pH 7.4. The drugs used in the experiment were AMPH, which was generously supplied by Dr. R. L. Walsh (Research Technology Branch, National Institute on Drug Abuse, USA), L-glutamate, N-methyl-D-aspartic acid (NMDA), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), D(-)-2-Amino-5-phosphonopentanoic acid (APV), forskolin and H89 (N-[2-(p-Bromo-cinnamylamino)ethyl]-5-isoquinolinesulfonamide) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Chelerythrine chloride was purchased from Tocris (Bristol, UK). Artificial cerebrospinal fluid, of identical volume to tested agents, was dispensed intrathecally to serve as a vehicle. At the end of the experiment, the location of the injection site was marked by an injection of 10 μl 2% Alcian blue. The volume of drug injected into the spinal cord in such experiments was reported to spread 0.5 to 1.5 mm from the site of injection (20). Therefore, a cannula positioned more than 0.5 mm from the intended site of injection was not included in the statistical analysis.

**Intrathecal (i.t.) Catheter**

An i.t. catheter was inserted as described by Yaksh and Rudy (28) and modified from Chen et al. (5). The occipital crest of the skull was exposed and the atlanto-occipital membrane was incised at the midline.
with the tip of an 18-gauge needle. A PE-10 catheter was inserted through the slit and passed caudally to the 6th lumbar level of the spinal cord. The volume of fluid within the cannula was kept constant at 10 μl in all experiments. Single 10 μl volume of drug solution was administrated, followed by a 10 μl flush of artificial cerebrospinal fluid (13). At the end of the experiment, a laminectomy was performed to verify the location of the cannula tip.

**PUR Activity**

The activities of PUR were assessed by recording the amplitude of action potentials and the spike numbers of external urethra sphincter electromyogram (EUSE) under test stimulation (TS; electric pulses at a frequency of 1/30 Hz, with intensity of 10 to 15 voltages) or repetitive stimulation (RS; frequency of 1 Hz with the same intensity of electric shock as TS) with/without i.t. application of AMPH, followed by administration of glutamate or NMDA, respectively.

**Experimental Arrangement**

The protocol for assessing the effects of electrical stimulation and different kinds of reagents on PUR activity was as follows: [1] TS: Once the electrodes’ positions were optimized, recording of EUSE activity was initiated. An electric current of square-wave pulse with pulse duration of 0.1 ms was applied from a stimulator (Grass S88, Cleveland, OH, USA) through a stimulus isolation unit (Grass SIU5B) and a constant current unit (Grass CCU1A). Electric shocks at fixed supra-threshold strengths (10 to 15 voltages) were repeated at interval of 30 seconds, and given through a pair of stimulation electrodes (referred to as TS). This frequency of stimulation was chosen for sampling data because it did not result in response facilitation. An electric intensity of stimulation that caused a single-spike action potential in the EUSE was recorded to standardize the baseline PUR activity. [2] RS: After the baseline period, RS at 1 Hz (lasting for 30 min for obtaining constant condition before agonist test) with an intensity identical to the TS (referred to as RS) was applied to induce facilitation in PUR. [3] Agonist test: After RS had been applied for 30 min, AMPH was used via the i.t. Six minutes after the application of AMPH, i.e., at a steady state of inhibition of PUR by AMPH, glutamate or NMDA was applied via the same route.

**EUS Electromyogram Activity**

Epoxy-coated copper wire (50 μm; M.T. Giken Co., Tokyo, Japan) electromyogram electrodes were placed in the EUS. This was performed using a 30-gauge needle with a hooked electromyogram electrode positioned at the tip. The needle was inserted into the sphincter approximately 1 to 2 mm lateral to the urethra and was then withdrawn, leaving the electromyogram wires embedded in the muscle. The activity of the external urethral sphincter electromyogram (EUSE) was amplified 20,000-fold and filtered (high frequency cut-off at 3,000 Hz and low at 30 Hz, respectively) by a preamplifier (Grass P511AC), then was continuously displayed on an oscilloscope (Tectronics TDS 3014, Wilsonville, OR, USA) and the recording system (Biopac, MP30, Santa Barbara, CA, USA). The stimulated nerve and the electrodes were bathed in a pool of warm paraffin oil (37°C) to prevent drying.

**Data Analysis**

Results were analyzed by the SPSS package (Statistical Package for the Social Sciences, SPSS/PC+; SPSS, Chicago, IL, USA). All the data are mean value ± SEM The statistical significances of the differences between various treatments and the pre-drug controls were determined by a two-tailed Student’s *t*-test by comparing control and experimental samples from different groups of preparations, and by the Student’s paired *t*-test by comparing control and experimental samples from the same groups of preparations. Differences are considered significant at *P* < 0.05.

**Results**

**Baseline Reflex Activity**

Reflex activity evoked by PAN TS (1/30 Hz) were obtained from 49 rats (see a typical example in Fig. 1A). A single-action potential was elicited by the TS. The mean latency for the TS to evoke the activity was 58.2 ± 8.4 ms (n = 49). The reflex activity varied little over a 10-min testing period (Fig. 1A). The mean spike numbers evoked by the TS at 10 min was 1.0 ± 0.0 spikes/stimulation (n = 49).

**RS-Induced Reflex Potentiation**

A spinal reflex potentiation (SRP) was induced by afferent nerve RS (Fig. 1B, 1 Hz) at the same intensity as the TS. The evoked firing increased gradually following the onset of the RS, then reached a plateau at about 10 min and maintained this level until the cessation of stimulation. As shown in Fig. 1B, mean spike numbers evoked by the RS, counted at 30 min following the RS onset, increased significantly (18.5 ± 0.5 spikes/stimulation *P* < 0.05, n = 49) compared with the baseline reflex activity induced by the TS (1.0 ± 0.0 spike/stimulation, Fig. 1C).
Agonist-Induced Potentiation

Intrathecal administration of glutamate (TS+GLU, 100 μM, 10 μl) and NMDA (TS+NMDA, 100 μM, 10 μl) both induced a SRP in TS-induced reflex activity, which was similar to the RS-induced reflex potentiation (Fig. 2A). Mean spike numbers induced by the TS with i.t. glutamate (TS+GLU, 21.7 ± 0.9 spikes/stimulation, \( P < 0.05, n = 10 \)) and by the TS with i.t. NMDA (12.0 ± 1.2 spikes/stimulation, \( P < 0.05, n = 10 \)) both increased significantly compared with the afferent nerve TS alone (Fig. 2C).

Antagonism Caused by CNQX and APV

i.t. administration of CNQX (100 μM, 10 μl) attenuated the RS-induced reflex potentiation. On the other hand, APV (100 μM, 10 μl) blocked the RS-induced reflex potentiation in the same preparations (Fig. 2B). The results showed that the mean spike numbers evoked by the afferent nerve stimulation, counted 30 min following stimulation onset, decreased significantly in both the RS with pretreated CNQX (7.4 ± 0.5 spikes/stimulation, \( P < 0.05, n = 10 \)) and in the RS with pretreated APV (1.1 ± 0.0 spikes/stimulation, \( P < 0.05, n = 10 \)) compared with the RS alone (20.7 ± 0.6 spikes/stimulation) (Fig. 2C).

The Effects of AMPH

Intrathecal administration of AMPH (TS+AMPH, 100 μM, 10 μl) induced a SRP in TS-induced reflex activity (Fig. 3A), which was similar to the RS-induced reflex potentiation. Mean spike numbers induced by the TS with i.t. AMPH (TS+AMPH, 17.3 ± 0.6 spikes/stimulation, \( P < 0.05, n = 10 \)) increased significantly compared with the afferent nerve TS alone (Fig. 3B).
Urine Retention Induced by D-Amphetamine

Effects of H89 on AMPH-Elicited SRP

Intrathecal administration of H89 (TS+H89, 10 μM, 10 μl) did not induce a SRP in TS-induced reflex activity and RS-induced reflex activity (Fig. 4). As shown in Fig. 4C, mean spike numbers induced by the TS with i.t. H89 (TS+H89, 1.0 ± 0.0 spikes/stimulation, \( P > 0.05, n = 10 \)) did not induce a SRP compared with the afferent nerve TS alone (1.0 ± 0.0 spikes/stimulation). Mean spike numbers evoked by the afferent nerve stimulation, counted 30 min following stimulation onset, did not decrease in the RS with pretreated H89 (RS+H89, 12.9 ± 0.9 spikes/stimulation, \( P > 0.05, n = 10 \)) compared with the RS alone (13.8 ± 1.2 spikes/stimulation).

Intrathecal administration of AMPH (TS+AMPH, 100 μM, 10 μl) induced a SRP in TS-induced reflex activity, further i.t. administration of H89 (TS+AMPH+H89, 10 μM, 10 μl) inhibited the AMPH-elicited SRP (Fig. 3A3). The mean spike numbers were decreased from 17.3 ± 0.6 spikes/stimulation (TS+AMPH, 100 μM, 10 μl) to 1.1 ± 0.1 spikes/stimulation (TS+AMPH+H89, 10 μM, 10 μl, \( P < 0.05, n = 10 \)) (Fig. 3B). Intrathecal pre-treated administration of H89 (10 μM, 10 μl) did not induce the AMPH (100 μM, 10 μl)-elicited SRP. As shown in Fig. 5B, mean spike numbers

Fig. 2. Baseline reflex activity and repetitive stimulation (RS)-induced reflex potentiation. A1: single-action potentials in the external urethra sphincter electromyogram (EUSE) were evoked by test stimulation (TS). A2: Glutamate (TS+GLU, 100 μM, 10 μl) induced reflex potentiation of TS-induced reflex activity. A3: NMDA (TS+NMDA, 100 μM, 10 μl) induced reflex potentiation of TS-induced reflex activity. B1: afferent nerve TS evoked a longer-lasting reflex potentiation. B2: afferent nerve TS evoked a longer-lasting reflex potentiation and were attenuated by CNQX (RS+CNQX, 100 μM, 10 μl). B3: afferent nerve RS evoked a longer-lasting reflex potentiation and were abolished by APV (RS+APV, 100 μM, 10 μl). C: mean spike numbers (±SEM, \( n = 10 \)) in reflex activity induced by TS, RS, by TS combined with glutamate (TS+GLU, 100 μM, 10 μl) or NMDA (TS+NMDA, 100 μM, 10 μl) as well as by RS combined with CNQX (RS+CNQX, 100 μM, 10 μl) or APV (RS+APV, 100 μM, 10 μl). ** and ***: \( P < 0.05 \), significantly different from TS (**) and RS (**), respectively.
induced by the TS (1.1 ± 0.1 spikes/stimulation, n = 10),
TS+H89 (1.3 ± 0.1 spikes/stimulation, n = 10), TS+
H89+AMPH100 (1.1 ± 0.1 spikes/stimulation, n = 10)
did not induce a SRP compared with the afferent nerve
TS alone (1.0 ± 0.0 spikes/stimulation, P > 0.05, n = 10).
It appears that H89 prevents and inhibits the AMPH-
elicted SRP in TS-induced reflex activity.

Effects of Chelerythrine Chloride on AMPH-Elicited
SRP

Mean spike numbers induced by the TS with i.t.
chelerythrine chloride (TS+CTC, 1.3 ± 0.1 spikes/
stimulation, P > 0.05, n = 10) did not induce a SRP,
compared with the afferent nerve TS alone (1.0 ± 0.1
spikes/stimulation) (Fig. 6A). Intrathecal administra-
tion of AMPH (100 μM, 10 μl) induced a SRP in the
presence of CTC in TS-induced reflex activity (TS+
CTC+AMPH100, 12.8 ± 1.1 spikes/stimulation). After
washing off CTC (10 μM, 10 μl) for 20 min, the SRP
still persisted in the presence of AMPH (TS+AMPH100,
15.1 ± 1.8 spikes/stimulation). Mean spike numbers
induced by the TS with i.t. CTC, AMPH (TS+CTC+
AMPH100, 12.8 ± 1.1 spikes/stimulation, P < 0.05, n =
10) increased significantly, compared with the afferent
nerve TS+CTC (Fig. 6B). It appears that CTC (10 μM,
10 μl) did not prevents AMPH (100 μM, 10 μl)-induced
SRP in TS-induced reflex activity.

The Effects of Forskolin

Intrathecal administration of lower concentra-
tions of forskolin (TS+FK10, 10 μM, 10 μl) did not
induce a SRP in TS-induced reflex activity (Fig.
7A2). Mean spike numbers induced by the TS with
intrathecal lower concentrations of forskolin (10 μM)
(TS+FK10, 1.0 ± 0.0 spikes/stimulation, P > 0.05, n =
10) did not increase significantly, compared with the
afferent nerve TS alone (1.0 ± 0.0 spikes/stimulation, Fig.
7D). However, intrathecal administration of higher
concentrations of forskolin (TS+FK30, 30 μM, 10 μl)
induced a SRP in TS-induced reflex activity (Fig.
7A3), which was similar to the RS-induced reflex
potentiation. Mean spike numbers induced by the TS
with intrathecal higher concentrations of forskolin
(TS+FK30, 6.3 ± 1.7 spikes/stimulation, P < 0.05, n =
10) increased significantly compared with the af-
ferent nerve TS alone (1.0 ± 0.0 spikes/stimulation,
Urine Retention Induced by $D$-Amphetamine

Intrathecal administration of lower concentrations of AMPH (TS+AMPH30, 30 μM, 10 μl) did not elicit SRP in TS-induced reflex activity (Fig. 7B2). Mean spike numbers induced by the TS with i.t. lower concentrations of AMPH (30 μM) (TS+AMPH30, 1.0 ± 0.0 spikes/stimulation, $P > 0.05$, $n = 10$) did not increase significantly, compared with the afferent nerve TS alone (1.0 ± 0.0 spikes/stimulation, Fig. 7D). However, i.t. administration of higher concentrations of AMPH (TS+AMPH100, 100 μM, 10 μl) induced a SRP in TS-induced reflex activity (Fig. 7B3), which was similar to the RS-induced reflex potentiation. Mean spike numbers induced by the TS with i.t. higher concentrations of AMPH (TS+AMPH100, 20.8 ± 4.2 spikes/stimulation, $P < 0.05$, $n = 10$) increased significantly, compared with the afferent nerve TS alone (1.0 ± 0.0 spikes/stimulation, Fig. 7D).

No SRP was observed at lower concentrations of forskolin (10 μM) treated preparation. It seemed that the lower concentration of forskolin did not elicit the SRP. AMPH at 30 μM did not elicit SRP. However, if AMPH (30 μM) was added to the spinal cord pre-treated with lower concentration of forskolin (10 μM), the SRP was found. The results showed that the facilitating effects of AMPH on the SRP elicited by AMPH were tested pre-treated with low concentrations of forskolin. Mean spike numbers induced by the TS with i.t. lower concentrations of forskolin (10 μM, 10 μl) and lower concentrations of AMPH (30 μM, 10 μl) (TS+FK10+AMPH30, 10.3 ± 1.2 spikes/stimulation, $P < 0.05$, $n = 10$) increased significantly compared with the afferent nerve TS alone (1.0 ± 0.0 spikes/stimulation, Fig. 7D).
Fig. 5. Effects of H89 and AMPH in TS-induced reflex activity. A1: single-action potentials in the EUSE were evoked by TS. A2: The effects of H89 (10 μM, 10 μl) in TS-induced reflex activity (TS+H89). A3: AMPH (100 μM, 10 μl) did not induce reflex potentiation of TS-induced reflex activity in the present of H89 (TS+H89+AMPH100). B: mean spike numbers (±SEM, n = 10) in reflex activity induced by TS, by TS combined with H89 (10 μM, 10 μl) (TS+H89) and by TS combined with AMPH (100 μM, 10 μl) and H89 (10 μM, 10 μl) (TS+H89+AMPH100).

Fig. 6. Effects of chelerythrine chloride (CTC) and AMPH in TS-induced reflex activity. A1: single action potentials in the EUSE were evoked by TS. A2: The effects of CTC (10 μM, 10 μl) in TS-induced reflex activity (TS+CTC). A3: The effects of AMPH (100 μM, 10 μl) in TS-induced reflex activity in the presence of CTC (TS+CTC+AMPH100). A4: AMPH (TS+AMPH100, 100 μM, 10 μl) induced reflex potentiation of TS-induced reflex activity after washing off CTC from A3. B: mean spike numbers (±SEM, n = 10) in reflex activity induced by TS, by TS combined with CTC (10 μM, 10 μl) (TS+CTC), by TS combined with CTC (10 μM, 10 μl) and AMPH (100 μM, 10 μl) (TS+CTC+AMPH100) and by TS combined with AMPH (100 μM, 10 μl) (TS+AMPH100). **: P < 0.05, significantly different from TS+CTC. ##: P < 0.05, significantly different from TS.
Discussion

Recent studies on PUR activities have shown that repetitive/tetanic stimulation of the pelvic afferent fibers induced a distinct and spinal SRP in PUR activities. This study demonstrated when compared with TS (1/30 Hz) evoked a baseline reflex activity with a single action potential, RS (1 Hz) induced SRP in the reflex activity. Intrathecal L-glutamate (100 μM, 10 μl) and NMDA (100 μM, 10 μl), both glutamate receptor...
agonists, induced excitatory effects on the TS-elicited reflex activity. Intrathecal CNQX (100 μM, 10 μl) and APV (100 μM, 10 μl), both also glutamate receptor antagonists, exhibited inhibitory effects on the RS-induced SRP (Fig. 2). These findings suggested that this stimulation-induced potentiation was glutamatergic NMDA receptor-dependent.

In this study, we investigated whether AMPH affected PUR plasticity and the possible neurotransmitters involved to clarify the effects of AMPH on mituration functions. Intrathecal AMPH (100 μM, 10 μl) induced excitatory effects on the TS (1/30 Hz) elicited reflex activity. And the excitatory effects induced by AMPH (100 μM, 10 μl) was inhibited by H89 (Fig. 3). H89 was commonly used as protein kinase A (PKA) inhibitors (17). H89 did not alter the SRP in TS and RS-induced reflex activity (Fig. 4). However, H89 prevented and inhibited the AMPH elicited SRP in TS-induced reflex activity (Figs. 3 and 5). We also used chelerythrine chloride (CTC), an inhibitor of cyclic AMP-dependent protein kinase C (10), to test the effect of protein kinase C (PKC) in AMPH (100 μM, 10 μl)-elicited reflex activity. In our study, the CTC (10 μM, 10 μl) did not prevents AMPH (100 μM, 10 μl)-induced SRP in TS-induced reflex activity. However, this stimulation-induced potentiation was glutamatergic NMDA receptor-dependent.

Forskolin, an activator of adenylyl cyclase (26), has been shown to activate the calcium current of the motor nerve terminal. In this study, we found that activation of adenylyl cyclase might facilitate the generation of the SRP elicited by AMPH. At the lower 30 μM concentration, AMPH did not elicit SRP. However, at this concentration, AMPH did elicit SRP if forskolin (10 μM, 10 μl) was added prior to the administration of AMPH (30 μM, 10 μl). However, a higher concentration of forskolin (30 μM, 10 μl) elicited SRP in TS-induced reflex activity (Fig. 7). These findings suggested that this stimulation-induced potentiation was glutamatergic NMDA receptor-dependent, and the obstructive bladder dysfunction induced by amphetamine addiction may be triggered by the PKA pathway activation.

Our findings suggested that extracellular application of amphetamine-like drugs induced reflex potentiation in NMDA-associated PUR leading to external urethral sphincter contraction, eventually achieving the urine continence via the PKA pathway. Results from this study may provide the basis for the development of treatment for amphetamine-associated urinary retention.

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