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INVOLVEMENT OF BRAIN GABAA RECEPTOR FOR ANGIOTENSIN II-INDUCED FREQUENT URINATION IN THE RAT

Hypothesis/aims of study

Psychological stress can lead to the exacerbation of urinary bladder dysfunction including overactive bladder and bladder pain syndrome. Recently, we showed that angiotensin II (Ang II), which is a stress-related neuropeptide, centrally increases urinary frequency by acting on brain Ang II type 1 (AT1) receptors without altering blood pressure [1]. However, the brain pathophysiological mechanisms underlying the Ang II induced micturition reflex still have not been clarified. Previous study showed brain Ang II can inhibit activity of the γ-aminobutyric acidergic (GABA)ergic nervous system [2], which has been also shown to regulate micturition reflex [3]. In the current study, we investigated central roles of $GABA_A$ receptor in the Ang II-induced frequent urination in the rat.

Study design, materials and methods

In urethane anesthetized (1.0 g/kg, ip) male Wistar rats (320-390 g), a catheter was inserted into the bladder dome in order to perform cystometry (12 ml/h saline infusion). Three hours after the surgery, Ang II (30 pmol/3 μl/rat, icv) or the vehicle (PBS) was centrally administered into the right ventricle (0.8 mm posterior and 1.5 mm right from the bregma, and 4.0 mm below the surface of the brain). Single or continuous cystometry was performed to calculate the voiding parameters before Ang II administration and from 0 to 60 min after the Ang II administration. When performing single cystometry, saline was infused into the bladder until the peak of a voiding bladder contraction, then the infusion was stopped and voided saline and residual urine from the bladder were collected and measured. Then, voided volume (VV), postovoiding residual urine volume (RV) were measured, and bladder capacity (BC), and voiding efficacy (VE) were calculated.

In the continuous cystometory, muscimol (Mus, $GABA_A$ receptor agonist, 100 or 300 pmol/3 μl/rat, icv) or vehicle (PBS) was administered 30 min before central Ang II (30 pmol/3 μl/rat) administration. Also, Ang II (100 or 300 pmol/300 μl/rat) was intravenously administered into the femoral vein. In the continuous cystometry, the intercontraction interval (ICI) and maximum voiding pressure (MVP) were evaluated 20 min before and after the Ang II administration.

Results

In the single cystometry, centrally administered vehicle failed to affect VV, RV, BC or VE compared to the pre-treatment (-20~0 min). While, centrally administered Ang II significantly reduced VV and BC without affecting RV or VE compared to the vehicle treated group for 0~60 min after the administration (Table 1). In the continuous cystometry, centrally administered Ang II significantly reduced ICI without affecting MVP compared to the vehicle-treated group for 0~60 min after the administration (Table 2). Centrally administered Mus alone failed to affect ICI or MVP compared to the vehicle treated group for 0~60 min. However, pre-treatment with high dose but not low dose of Mus significantly suppressed the Ang II induced reduction in ICI compared to the vehicle for 0~40 min after the administration (Table 3). On the other hand, intravenously administered Ang II failed to affect ICI or MVP for 0~60 min after the injection compared to the pre-treatment (-20~0 min).

Interpretation of results

Current data demonstrated that pre-treatment with Mus suppressed Ang II-induced micturition reflex. GABA is an important inhibitory neurotransmitter in the nervous system. And, $GABA_A$ receptor agonist is known to inhibit the micturition reflex in the brain [3]. There are several findings shows about a relationship between Ang II and GABAergic nervous system in the brain [2]. AT1 receptors and $GABA_A$ receptors have been shown to appear within the periaqueductal gray (PAG), a site coordinating activity of the pontine micturition center. Previous report showed Ang II-induced stimulation of AT1 receptors inhibits GABA release in the PAG [2]. These data suggest that brain Ang II increases frequent urination via inhibition of GABAergic nervous system in the brain.

Concluding message

The brain Ang II nervous system is involved in the facilitation of the rat micturition through the inhibition of the GABAergic nervous system, specifically involved in $GABA_A$ receptors.

Table 1. Effects of centrally administered Ang II on urodynamic parameters in single cystometry

	VV (%)		RV (%)		BC (%)		VE (%)	
Timing of evaluation	% of Control							
Icv administration	Vehicle	Ang II	Vehicle	Ang II	Vehicle	Ang II	Vehicle	Ang II
0~20 min	103.8 ± 9.2	56.8 ± 6.3*	96.8 ± 7.0	90.6 ± 8.3	100.0 ± 6.5	70.9 ± 6.2*	102.5 ± 5.8	90.5 ± 6.3
20~40 min	101.8 ± 5.8	64.9 ± 3.1*	91.8 ± 8.3	94.1 ± 9.5	96.5 ± 6.3	72.7 ± 3.9*	105.4 ± 4.8	88.1 ± 4.0
40~60 min	106.9 ± 9.0	77.4 ± 4.9*	87.6 ± 9.3	86.4 ± 10.4	93.7 ± 4.7	81.7 ± 4.3*	114.4 ± 9.0	95.5 ± 7.5

Data were calculated as the ratio to the control values before icv administration (-20~0 min) showed as means ± SEM. *P <0.05 (compared with the vehicle-treated group by the unpaired Student's t-test). Vehicle: PBS (3 μl) (n = 5), Ang II: Angiotensin II 30 pmol/3 μl (n = 8). VV: voided volume, RV: post voiding residual urine volume, BC: bladder capacity, VE: voiding efficacy.

Table 2. Effects of centrally administered Ang II on urodynamic parameters in continuous cystometry

	ICI (%)		MVP (%)	
Timing of evaluation	% of Control			
Icv administration (0 min)	Vehicle	Ang II	Vehicle	Ang II
After icv administration (0~20 min)	101.6 ± 3.8	66.1 ± 3.6*	101.1 ± 2.0	109.7 ± 4.1
After icv administration (20~40 min)	105.4 ± 2.3	66.0 ± 4.6*	99.9 ± 2.4	107.9 ± 3.7
After icv administration (40~60 min)	101.2 ± 1.3	68.6 ± 4.6*	100.8 ± 2.3	101.5 ± 4.5

ICI: intercontraction interval, MVP: maximum voiding pressure. Data were calculated as the ratio to the control values before icv administration (-20~0 min) showed as means ± SEM. *P <0.05 (compared with the vehicle-treated group by the unpaired Student's t-test). Vehicle: PBS (3 μl) (n = 6), Ang II: Angiotensin II 30 pmol/3 ul (n = 6).

Table 3. Effects of pre-treatment with $GABA_A$ agonist, muscimol on centrally administered Ang II induced-change of urodynamic parameters in continuous cystometry

	ICI (%)		
Timing of evaluation	% of Control		
1st icv administration (-30 min)	Vehicle	Mus100	Mus300
2nd icv administration (0 min)	Ang II	Ang II	Ang II
After Ang II administration (0~20 min)	66.0 ± 1.2	76.7 ± 5.5	90.3 ± 2.8†
After Ang II administration (20~40 min)	69.9 ± 2.6	80.7 ± 5.9	86.1 ± 5.1†
After Ang II administration (40~60 min)	76.1 ± 2.1	82.4 ± 4.2	87.0 ± 7.7

Vehicle: PBS (3 μl), Mus100: muscimol 100 pmol (3 μl), Mus300: muscimol 300 pmol (3 μl), Ang II: Angiotensin II (3 μl). Data were calculated as the ratio to the control values before Ang II administration showed as means ± SEM. †P <0.05 compared with the vehicle-pretreated group by the Fisher's PLSD method. Muscimol or vehicle was administered 30 min before central Ang II administration. Vehicle + Ang II (n = 6), Mus100 + Ang II (n = 7), Mus300 + Ang II (n = 6).

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Disclosures

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