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GABAERGIC MECHANISM MEDIATED VIA D1 RECEPTORS IN THE RAT PERIAQUEDUCTAL GREY PARTICIPATES IN MICTURITION REFLEX: AN IN VIVO MICRODIALYSIS STUDY

Hypothesis / aims of study

A micturition reflex is known to be regulated by a complex control system mediated via several neurotransmitters. Much interest has focused on the dopaminergic (DAergic) regulation, based on the previous studies showing that central administration of DAergic agents influenced the bladder activity[1].

Recently, a PET study revealed that the periaqueductal gray (PAG) is the crucial brain region in pathophysiology of lower tract dysfunction observed in Parkinson's disease, which is characterized by nigrostriatal DA depletion[2]. It leads us to suppose that the PAG DA neuronal system plays a significant role in mediating micturition reflex, however few studies have addressed about its detailed mechanisms. Here in, we neurochemically investigated the functional role of PAG DA neuron in mediating micturition reflex underlying modulation of neurotransmitters in freely moving rats. For this purpose, an *in vivo* microdialysis was used to examine dynamic changes in DA, glutamate and GABA in the PAG, focusing on the following points: 1) micturition reflex under physiological conditions and 2) pharmacological intervention by application of DAergic agents. To further clarify the mechanism by which DAergic modulation, experiment was carried out in 6-OHDA-lesioned rats, an animal model of Parkinson's disease.

Study design, materials and methods

Male Wistar rats (10-13 weeks) were used. Under anesthesia, a dialysis guide cannula was stereotaxically inserted into the PAG (lateral, 0.8 mm; posterior, 7.8 mm; ventral, -6.8 mm from bregma and dural surface) and a bladder catheter (PE-50) was in place. Three days after surgery, a dialysis probe was inserted through the guide cannula and perfused with artificial cerebrospinal fluid at a flow rate of 2 µl/min. Samples were collected at 20 min, and dialysate neurotransmitters were measured by high performance liquid chromatography. Throughout the microdialysis experiment, saline was infused into the bladder through a catheter at a rate of 0.2 ml/min for 20 min. Bladder function was evaluated by simultaneous measurement of intercontraction interval (ICI) and maximal voiding pressure (MVP) in cystometrograms (CMG). Drugs were applied locally into the PAG through the dialysis probe. To lesion DA neurons, 6-OHDA (8 µg) was unilaterally microinjected into the left substantia nigra pars compacta (SNc) (lateral, 2.2 mm; posterior, 5.3 mm; ventral, -8.0 mm from bregma and dural surface)[3]. Two weeks later, microdialysis experiments were performed in combination with physiological approaches using CMG in a similar manner. Sham operations were performed by vehicle injection in SNc. After the experiments, the lesions induced by 6-OHDA were confirmed by measuring DA contents.

Results

Extracllular levels of DA and glutamate in the PAG were increased with infusion of saline, whereas GABA levels were decreased in micturition reflex (Fig. 1). With continuous infusion of saline in the bladder, reproducible changes in neurotransmitters and micturition patterns were observed. As shown in Fig. 1, increases in both DA and glutamate levels induced by micturition were not altered, whereas GABA levels were increased in the presence of D_1 antagonist. The D_1 antagonist (SCH 23390, 10 µM) applied into the PAG caused reduction of ICI (3.55±0.64 min) and increase of MVP (43.1±5.1 cm water) compared with controls (ICI:4.78±0.65, MVP:25.8±3.6) (Fig. 2). Neither D_2 antagonist (remoxipride, 10 µM), D_1 agonist (SKF 38393, 10 µM) nor D_2 agonist (quinpirole, 10 µM) affected such physiological parameters (data not shown).

In 6-OHDA-lesioned rats, the ICI with infusion of saline were significantly decreased as compared to sham-operated rats (6-OHDA: 2.53±0.36 min, sham: 4.23±0.33 min: $p<0.05$). Glutamate levels were also increased by micturition, GABA levels were not decreased but increased in 6-OHDA-lesioned rats (Fig. 3, 4).

Interpretation of results

This is the first attempt that examines dynamic changes in neurotransmitters in the PAG during the micturition reflex in freely moving rats. The essential findings are that extracellular levels of DA and glutamate in the PAG were increased, whereas GABA levels were decreased in parallel with micturition. These results suppose that the DAergic and glutamatergic neurons contribute to the excitatory modulation of micturirion reflex while GABAergic neurons are involved in the inhibitory one. Furthermore, application of D_1 receptor antagonist into the PAG induced facilitation of micturition reflex and inhibition of decreases in GABA levels. Thus, PAG GABAergic neurons appear to be under tonic DAergic control, in turn, GABAergic mechanism mediated via D_1 receptor might be responsible for the micturition reflex. This hypothesis was supported by the finding that depletion of DA facilitates the micturition reflex. Furthermore, we observed that PAG GABA was not decreased but increased by micturition in 6-OHDA-lesioned rats. These findings suggest that at least in the PAG, reduction in GABAergic tone underlying DAergic modulation may participate in lower urinary tract dysfunction in Parkinson's disease.

Concluding message

The present study suggests that GABAergic mechanism mediated via D_1 receptors in the rat PAG contributes to micturition reflex. The bladder dysfunction of Parkinson's disease could be attributed to the derangement of this regulatory mechanism.

References

1. Neurourol Urodyn (2001) 20; 105-113.
2. J Urol (2006) 175; 994-998.
3. Br J Pharmacol (2003) 139; 1425-1432.

Fig.1: Area Under the Curve (AUC) of extracellular levels of neurotransmitters dynamic changes of neurotransmitters induced by micturition reflex with and without D1 antagonist (each n=6)

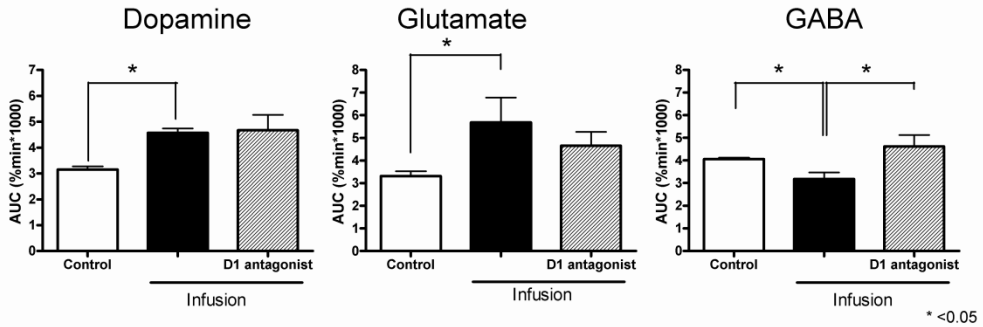


Fig.2: Original recordings of cystometrogram before and after D1 antagonist administration

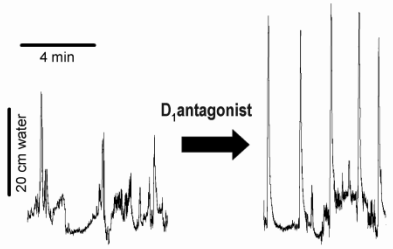


Fig.3: Original recordings of cystometrogram intact and 6-OHDA lesioned rat

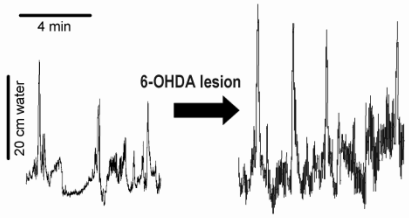
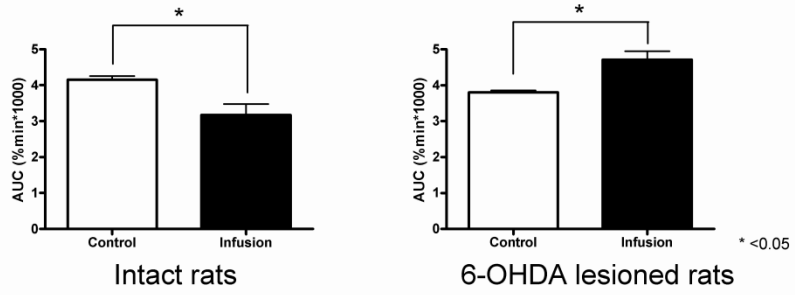


Fig.4: Area Under the Curve (AUC) of extracellular levels of GABA dynamic changes of neurotransmitters induced by micturition reflex intact and 6-OHDA lesioned rat (each n=6)



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ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by Hokkaido University Institutional Animal Care and Use Committee