

ACTIVATION OF THE NUCLEUS RETICULARIS PONTIS ORALIS INHIBITS MICTURITION AND CHANGES GLUTAMATE AND GLYCINE LEVELS IN THE LUMBOSACRAL CORD OF THE RATS

Aims of Study

In the central nervous system, glutamate is considered to be the major excitatory neurotransmitter and facilitates the micturition reflex (1). On the other hand, glycine is the most abundant inhibitory neurotransmitter in the brainstem and the spinal cord (2). However, there are little known about the inhibitory mechanism of the glycine neurons to the micturition reflex. Recently, it has been reported that injection of cholinergic agent into the rostral pontine reticular formation induced atonia and the increase of the glycine level in the spinal cord in cats (3). According to this area and micturition, it has been reported that the injection of carbamylcholine chloride (carbachol) or flavoxate hydrochloride (flavoxate) into the nucleus reticularis pontis oralis (PoO) inhibited bladder contractions in cats, and that neurons in the PoO activated during collecting phase in cats (4,5). Therefore, in this study, we examined which chemical agents injected into the PoO could influence bladder activity, and whether the change of bladder activity and the changes of amino acid levels in the lumbosacral cord by chemical stimulation of the PoO would be in agreement in rats.

Methods

Forty-four female Sprague-Dawley rats were used. The rats were divided into two groups; one group for cystometry and another group for amino acid analysis. All rats were anesthetized by urethane, and a small hole was made in the cranial bone (bregma -9.5 mm, R 1.0 mm). In twenty rats of cystometry group, a polyethylene catheter (PE-50) was inserted into the bladder through the urethra. The urethra was ligated to the catheter near the external urethral meatus, and bladder activity was monitored via the urethral catheter that was connected to a pressure transducer and a saline-infusion pump through a three-way stopcock. The bladder was filled with physiological saline (0.05 ml/min) to above the threshold volume, inducing isovolumetric rhythmic contractions. After the bladder contractions had become stable for over 30 min, 0.5 µl of physiological saline, carbachol, flavoxate, serotonin, and noradrenalin (1-10 mg/ml, each n = 4) were injected into the PoO by a microsyringe, and the change of bladder activity was recorded. In twenty-four rats of amino acid analysis group, 0.5 µl of physiological saline, carbachol, flavoxate, serotonin, and noradrenalin (1-10 mg/ml, each n = 4) were injected into the PoO, and these rats were sacrificed at 3-10 min after injection. The lumbosacral cord was harvested from these rats and intact control rats (n = 4). The tissue was homogenized with 0.5 M hydrochloric acid, and the supernatant was dechlorinated and deproteinized. Then, glutamate and glycine levels were measured by capillary electrophoresis system. Data were expressed as means ± standard deviation.

Results

When the interval (2.5 ± 0.9 min), amplitude, duration of bladder contractions, and the baseline pressure (18.7 ± 2.7 cm H₂O) became stable, injection of physiological saline did not influence any parameters of bladder contractions and the baseline pressure. When carbachol was injected, bladder contractions disappeared and the baseline pressure was gradually and significantly ($p = 0.016$) increased (69 % increase) over 30 min. Injection of flavoxate also transiently abolished bladder contractions, but bladder contractions completely recovered at 8-18 min. Injection of serotonin also gradually and significantly ($p = 0.039$) increased (27 % increase) the baseline pressure until 10 min after injection. There were no significant changes of parameters of bladder contractions after injection of noradrenalin. After injection of physiological saline into the PoO, the glutamate and glycine levels of the lumbosacral cord were 11.1 ± 3.5 µmol/g.tissue and 0.5 ± 0.3 µmol/g.tissue, respectively. These glutamate and glycine levels of the lumbosacral cord were not different from those in intact rats. When carbachol was injected, both glutamate (12.2 ± 0.5 µmol/g.tissue) and glycine levels (5.1 ± 0.6 µmol/g.tissue) were significantly ($p = 0.028$, $P = 0.006$, respectively) increased. Injection of flavoxate significantly ($p = 0.005$) increased the glycine level (4.5 ± 0.6 µmol/g.tissue), and

injection of serotonin significantly ($p = 0.046$) increased the glutamate level (12.1 ± 0.5 $\mu\text{mol/g.tissue}$). However, injection of noradrenerlin did not influence these amino acids levels.

Conclusions

In the present study, injections of carbachol and flavoxate abolished bladder contractions, and increased the glycine level in the lumbosacral cord. Injections of carbachol and serotonin increased the baseline pressure, and increased the glutamate level in the lumbosacral cord. Since the baseline pressure gradually increased after injections of carbachol and serotonin, this effect might be due to diffusion of these agents to adjacent regions. However, the changes of bladder activity and amino acid levels in the lumbosacral cord were in agreement in rats. PoO neurons activate lumbosacral glycine neurons which inhibit bladder activity.

References

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