

NORADRENALINE RELEASES FROM RAT URINARY BLADDER USING IN VIVO MICRODIALYSIS PROCEDURE

Aims of Study

Micturition cycle are divided into the storage and voiding phases. It has been suggested that in the storage phase, inhibition of bladder contractions was mediated by noradrenaline through the beta-adrenoceptors. However, there were few reports about noradrenaline releases from urinary bladder smooth muscles. In this study, we attempted to measure the noradrenaline releases from rat bladder in vivo, and evaluate the relationship between the amount of noradrenaline releases and the change in the bladder volume using in vivo microdialysis technique.

Methods

Female wistar rats (300-350 g) were used in the present studies. All rats were anesthized with 0.9 mg/kg urethane, and the lower abdominal cavity was opened with a mid-line incision, the bilateral ureters were ligated and dissected, and a 20 G cannula was inserted into the bladder transurethrally and physiological saline was infused into the urinary bladder. Bladder volumes are adjusted by the infused volume of physiological saline (0, 0.5 and 10 ml). In another experiment, the pelvic nerves of the latter group rats were sectioned bilaterally at the central end of the pelvic plexus. The peripheral end of one of the pelvic nerves was placed on a bipolar platinum electrode. Electrical field stimulation (EFS) on pelvic nerve (supramaximum voltage, pulse duration 0.1 msec, frequency 1-10 Hz, train of pulse 60s) was performed. In both experiments, the microdialysis probe (O-P-30-5, Eicom Co., Kyoto, Japan) was inserted into the smooth muscle of the bladder wall and the inlet cannula of the probe was connected to a microinfusion syringe pump (EP-60, Eicom Co.). Ringer solution (NaCl 147 mM, KCl 4 mM, CaCl₂ 2.3 mM, the pH was adjusted to 7.4 with containing 50 µM ascorbic acid was continuously perfused at a rate of 2 µl/min. In the bladder volume changing-experiment, sampling was started at 20 min after changing of the bladder volume and dialysate was collected in a microtube for 10 min, and a volume of 10 µl was injected into the noradrenaline assay system. In the EFS-experiment, sampling was started at 10 min before stimulation and dialysate was collected in a microtube every 10 min, and a volume of 10 µl was injected into the noradrenaline assay system. The amount of noradrenaline in the dialysate fraction was measured by HPLC with ECD as previously reported (1). We evaluated the relationship between the amount of noradrenaline releases from urinary bladder and the change in bladder volume. Furthermore, the effects of EFS of pelvic nerves on noradrenaline release from rat bladder was evaluated.

Results

According to increase in bladder volume, amounts of noradrenaline releases from rat bladder were gradually increased. The noradrenaline releases at 1.0 ml bladder volume was 0.080±0.012 pmol/injection, which was significantly higher than that of 0 ml in bladder volume (0.039±0.015 pmol/injection).

The amounts of noradrenaline releases in EFS of pelvic nerve at 10 Hz and without EFS on pelvic nerve were 0.047±0.021 and 0.045±0.015 pmol/injection, respectively. There were not significant difference between the values.

Conclusions

The present study demonstrated that in vivo microdialysis procedure are useful method for measurement of noradrenaline release from rat bladder. The findings of a significant bladder volume-related increase in noradrenaline releases, may suggest that the increased noradrenaline releases contribute to regulation of bladder tone in the filling phase.

References

1. Eur. J. Pharmacol., 357, 213-219, 1998

Table 1: The noradrenaline release in different bladder volume

Bladder volume	0 ml	0.5 ml	1.0 ml
Noradrenaline release (pmol/injection)	0.039	0.052	0.080*

Noradrenaline releases measured by in vivo microdialysis method

* $p < 0.05$: significantly different from the values of bladder volume

Table 2: The noradrenaline release in EFS on pelvic nerve

EFS	Before EFS	1 Hz	10 Hz
Noradrenaline release (pmol/ injection)	0.047	0.040	0.045

Without significantly different from the values of bladder volume