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MEASUREMENT OF ACETYLCHOLINE RELEASED FROM RAT DETRUSOR SMOOTH MUSCLE USING IN VIVO MICRODIALYSIS

AIMS OF STUDY

The parasympathetic nervous system plays an important role in the function of the lower urinary tract. A major neurotransmitter for physiological bladder contraction is acetylcholine released from prejunctional parasympathetic nerve endings. Microdialysis is a relatively new sampling technique that has been extensively applied in neurotransmitter measurement. We had previously demonstrated that high performance liquid chromatography with electrochemical detection coupled with microdialysis procedure was applicable to the direct determination of the basal release of acetylcholine as well as the acetylcholine evoked by electrical field stimulation from smooth muscle strips of rabbit detrusor in vitro (1, 2, 3) However, there is little information yet available on the in vivo measurement of acetylcholine released from detrusor smooth muscle Therefore, we measured acetylcholine released from rat detrusor smooth muscle induced by pelvic nerve stimulation using high performance liquid chromatography coupled with in vivo microdialysis procedure.

METHODS

Female Sprague-Dauley rats, weighing from 200 to 250 g, were anesthetized with urethane (900 mg/kg, SC). A 20 G cannula was inserted into the bladder transurethrally, and was connected to a pressure transducer The microdialysis probe was inserted into the bladder wall and was continuously perfused with a Ringer solution containing physostigmine sulphate at a rate of 2 μl/min. The dialysate was collected every 10 min The pelvic nerves 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 and the nerve was stimulated for 60 sec, 5 times at 1 min intervals, with an electrical stimulator at 1-10 Hz, with a supramaximum voltage with a square wave of a 1 msec duration The acetylcholine content of the dialysate was determined by high performance liquid chromatography coupled with an electrochemical detector. In addition, we investigated the effect of atropine (0 05-0.1 mg/kg, V)on bladder contraction and acetylcholine release induced by pelvic nerve stimulation

RESULTS

In the present experimental conditions, the pelvic nerve stimulation caused bimodal contractions of the bladder. The initial phase was a rapidly rising contraction, and the plateau phase was a tonic contraction. There were frequency dependent increases in both phase contractions. The detection limit of acetylcholine was about 10 fmol/injection. The value for acetylcholine released from detrusor smooth muscle before stimulation was 36 ± 6 fmol/injection. Acetylcholine releases induced by pelvic nerve stimulation increased in a frequency dependent manner. Treatment with atropine concentration dependently suppressed the plateau phase, however, initial phase contraction were not significantly affected by atropine even at higher dose. Atropine had no significant effects in acetylcholine release.

CONCLUSIONS

The present study demonstrated that pelvic nerve stimulation caused frequency dependent bimodal contraction and acetylcholine release in rat bladder. Atropine caused a decrease in contraction especially in a plateau phase, but had not significant effect in acetylcholine release. The data suggest that this method is useful to investigate both acetylcholine release from rat bladder and bladder contraction in vivo Furthermore, using this technique, it may be possible to evaluate the actions of various neurotransmitters on bladder function in vivo.

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