118

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EFFECTS OF INTRAVESICAL CAPSAICIN, RESINIFERATOXIN AND ATP ON PRIMARY BLADDER AFFERENT ACTIVITY OF THE RAT

Hypothesis / aims of study

Previous studies have suggested that increased extracellular ATP has a role in mechano-afferent transduction in the rat bladder and that ATP-induced facilitation of the micturition reflex is mediated, at least partly, by nerves other than capsaicin (Cap)-sensitive afferent nerves (1). This study aimed to investigate the direct effect of intravesical administration of ATP on A-delta- and C-fiber activities and to determine its relationship with Cap-sensitivity.

Study design, materials and methods

Female Sprague-Dawley rats were used. After intraperitoneal urethane anesthesia (0.9 g/kg), the left pelvic nerve was identified and put on an electrode for electrical stimulation. A catheter (PE-50) was inserted into the bladder dome for filling and emptying of the bladder. The lumbo-sacral spinal cord was exposed by laminectomy and bilateral L6 dorsal roots were cut. The dorsal skin was tied up to make a pool and spinal cord was covered with body warm paraffin oil. Fine filaments were dissected from the left L6 dorsal root and placed on recording electrodes. Afferent fibers originating from the bladder were identified by electrical stimulation of the pelvic nerve and by bladder filling. Those with a conduction velocity (CV) <2.5 m/sec were considered to correspond to C-fibers and those with a CV >2.5 m/sec to A-delta-fibers (2). To facilitate permeability of the bladder urothelium for drugs, protamine sulfate solution (10 mg/ml, 0.3 ml) was instilled intravesically and kept in the bladder for 60 minutes just before the afferent activity measurement. Afferent activity was studied during constant filling cystometry at 0.08 ml/min until an intravesical pressure of 30 cmH_2O was reached. At the beginning of the experiments, three cystometric investigations by constant filling with saline were performed, and the third cystometry served as the control observation for the following fillings. Then, Cap (10^{-5} M, 0.1 ml, for 2 min), resiniferatoxin (RTX; 10^{-6} M, 0.3 ml, for 30 min to desensitize Cap-sensitive nerves), and Cap (10^{-5} M, 0.1 ml, for 2 min) again were administered intravesically. Thereafter, ATP 10^{-3} M or its vehicle was instilled to obtain another three cystometric investigations (Figure 1). The afferent activity is expressed as a percentage of baseline activity, integrated for the whole filling phase.

Results

Sixteen single units of primary bladder afferent fibers were isolated in fifteen rats. Eleven units fulfilled the criteria for C-fibers (CV: 1.500 ± 0.077 m/sec), and five for A-delta-fibers (CV: 4.047 ± 0.478 m/sec). For the A-delta-fibers, a slightly increased afferent activity was observed just after instillation of RTX (Figure 2A) in association with decreased intravesical pressure (5 to 10 cmH_2O) at the first filling cystometry with the vehicle. However, total afferent activities with instillation of the vehicle were not significantly different from the base-line (base-line: 100%, and 1st, 2nd, and 3rd instillation of vehicles: 117%, 117%, and 118%, respectively). For the C-fibers, two types of afferent activities were observed; one was Cap-insensitive, and characterized as no response to intravesical Cap (Figure 2B), the other one was Cap-sensitive, and characterized as positive responses to the first instillation of Cap and RTX, but no response to the second instillation of Cap (Figure 2C). Ten single-units of the Cap-insensitive afferent fibers were isolated, and the activities of these Cap-insensitive fibers in response to the bladder filling did not change significantly when filled with the vehicle (n=5), but significantly increased with ATP (n=5) at the 1st instillation (Figure 3). Only one single unit of the Cap-sensitive fiber was isolated, and this fiber did not respond to the instillation of ATP.

Interpretation of results

The results of the present study suggest that mechano-sensitive A-delta fibers were not affected by intravesical Cap or RTX. On the other hand, there are two types mechano-sensitive C-fibers of the primary rat bladder afferents, Cap-sensitive and -insensitive, the latter predominating. Furthermore, the present results also suggest that intravesically applied ATP can facilitate Cap-insensitive, mechano-sensitive C-fibers among the three subsets of the primary bladder afferent nerves. Further investigations will be needed to determine the effects of intravesical ATP on the A-delta fibers.

Concluding message

Mechano-sensitive primary afferents of the rat bladder can be classified as A-delta, capsaicin-insensitive and capsaicin-sensitive C-fibers. The activation of the primary bladder afferents induced by intravesical instillation of ATP is mediated mainly through a subset of capsaicin-insensitive C-fibers.

References

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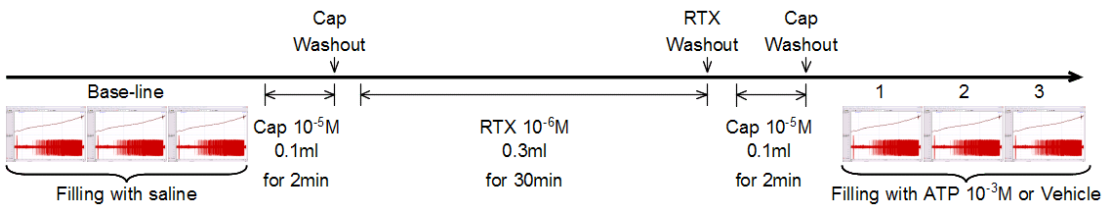

Figure 1. Experimental protocol.
Cap: Capsaicin, RTX: Resiniferatoxin, ATP: Adenosine 5'-triphosphate

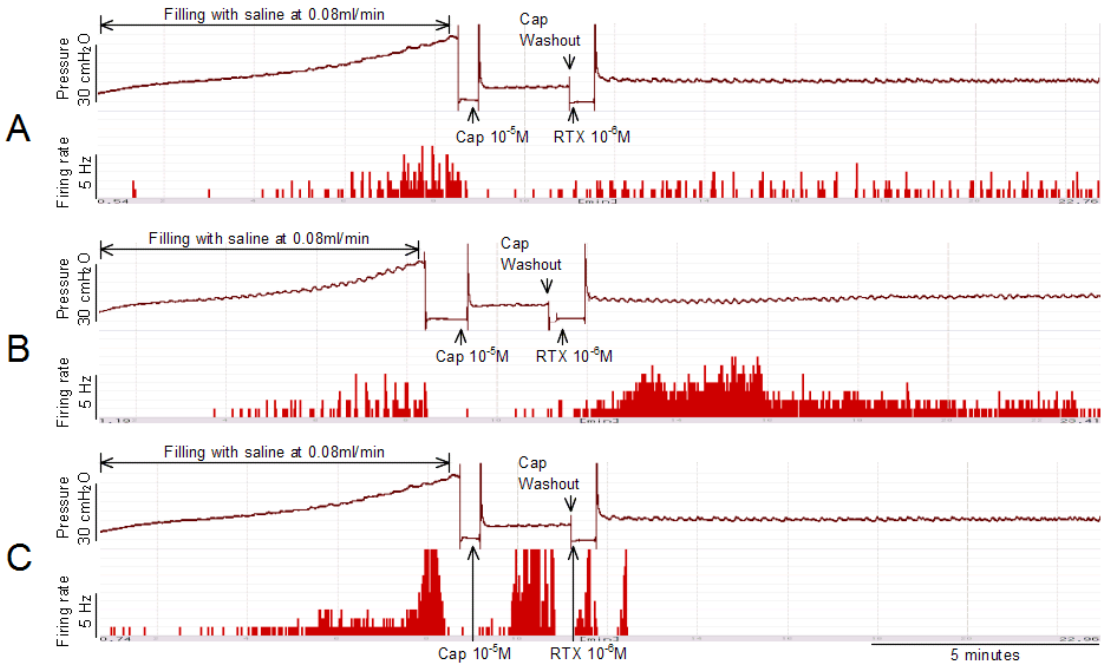

Figure 2. Intravesical pressure (upper trace) and single unit of primary bladder afferent activities (lower trace) during filling with saline and instillation of Cap/RTX.
A: A-delta-fiber. B: Cap-insensitive C-fiber. C: Cap-sensitive C-fiber.

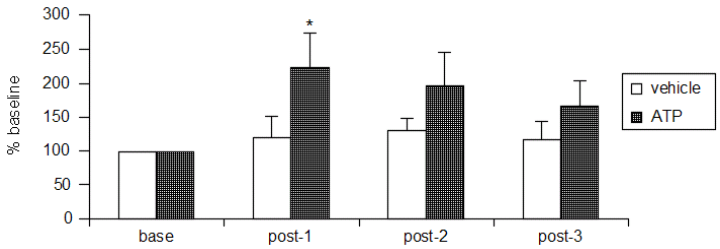

Figure 3. Response of the Cap-insensitive C-fibers to intravesical instillation of ATP or vehicle integrated during the whole filling phase. n=5 in each group,*p<0.05 versus base-line.

Specify source of funding or grant	**No funding or grant**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Animal Ethics Committee of Shinshu University**