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EFFECTS OF INTRAVESICAL TNP-ATP, A P2X3 PURINOCEPTOR ANTAGONIST, ON MICTURITION AND ATP-INDUCED BLADDER OVERACTIVITY IN CONSCIOUS RATS

Aims of study

The urothelium is known to release adenosine triphosphate (ATP) by stretch stimuli such as bladder distension. Recent studies using knockout mice provide evidence for a major role for urothelially released ATP, acting via the $P2X_3$ ($P2X_{2/3}$) purinoceptors on a subpopulation of pelvic afferent nerves, in the mechano-afferent transduction of the bladder [1]. Intravesical instillation of ATP causes bladder overactivity in conscious rats [2, 3], which can be blocked by systemic administration of TNP-ATP, a selective $P2X_3$ purinoceptor antagonist [2]. To elucidate the role of $P2X_3$ purinoceptors on bladder suburothelial afferent nerves in the physiological control of micturition threshold, in the present study, we investigated the effect of intravesical instillation of TNP-ATP on micturition in conscious rats. We also determined whether pretreatment with intravesical TNP-ATP can block the bladder overactivity induced by intravesical ATP in conscious rats.

Materials and methods

Female Sprague-Dawley rats, weighing 220-250g, were used. A polyethylene catheter (PE-50) was implanted into the bladder through the bladder dome. Cystometric investigations were performed without any anaesthesia 3 days after the bladder catheter implantation. Saline was instilled into the bladder at 10ml/h by a syringe infusion pump. Intravesical pressure and micturition volumes were continuously recorded. When the base-line cystometric investigations were obtained with saline instillation, saline containing protamine (10mg/ml) was instilled intravesically by changing the syringe of the infusion pump, and 0.5ml of the protamine solution was kept in the bladder for 60 min to facilitate the drugs, subsequently administered intravesically, to reach the bladder suburothelial layer since protamine is known to increase the permeability of the urothelium. Then, all the rats were randomly assigned to two groups. In one group of rats, cystometric investigations were again performed with saline instillation for 30 min, and then with saline containing ATP (0.1mM), or TNP-ATP (0.5μM or 5.0μM) for another 60 min. In the other group of rats, cystometric investigations were carried out with intravesical instillation of saline containing TNP-ATP (0.5μM) for 30 min. Then TNP-ATP (0.5μM) was switched to a combined solution of TNP-ATP (0.5μM) and ATP (0.1mM), and cystometric investigations were continued for another 60 min. The following cystometric parameters were investigated: basal pressure, threshold pressure, micturition pressure, voiding interval, micturition volume, residual volume, and bladder capacity during 3 micturition cycles before and after each drug administration. The results are given as mean values±standard deviations. A Student's paired two tailed *t*-test was used for comparisons before and after each intravesical drug administration. A probability level of <5% was accepted as significant.

Results

Intravesical instillation of protamine itself did not affect any cystometric parameters [data not shown]. In the protamine-pretreated rats, intravesical instillation of TNP-ATP(0.5μM and 5.0μM) dose-dependently induced significant increases in threshold pressure, voiding interval, micturition volumes and bladder capacity [Table 1]. Intravesical instillation of ATP (0.1mM) induced significant decreases in threshold pressure, voiding interval, micturition volumes and bladder capacity [Table 1]. The onset times of these effects of both TNP-ATP and ATP were about 5 min. In the presence of TNP-ATP(0.5μM), ATP (0.1mM) did not affect any cystometric parameters [Table 1].

Interpretation of results

The present findings that intravesical administration of the $P2X_3$ purinoceptor antagonist, TNP-ATP, induced increases in the threshold pressure and threshold volume, as indicated an increase in bladder capacity, for micturition during bladder filling, support the view that $P2X_3$

purinoceptors are involved in the physiological control of micturition threshold during the filling phase. Moreover, we confirmed that ATP, given intravesically, induces bladder overactivity, and that the ATP-induced bladder overactivity can be blocked by the pretreatment with intravesical TNP-ATP. The action sites of both ATP and TNP-ATP are most probably the $P2X_3$ purinoceptors on the bladder suburothelial afferent nerves, since the effects of both the two drugs were pronounced and the onset of the effects was quicker in protamine-pretreated rats than that in rats without protamine-pretreatment in previous studies[3 & unpublished data].

Concluding message

All together, the present results strongly suggest that ATP released from the bladder urothelium during bladder filling, acting via $P2X_3$ purinoceptors, play a physiological role in the mechano-afferent transduction of the bladder.

References

1. $P2X_3$ knock-out mice reveal a major sensory role for urothelially released ATP. J Neurosci, 21: 5670-5677, 2001.
2. Intravesical adenosine-triphosphate stimulates the micturition reflex in awake, freely moving rats. J Urol,168: 1230-1234, 2002.
3. Effects of resiniferatoxin desensitization of capsaicin-sensitive afferents on detrusor overactivity induced by intravesical capsaicin, acetic acid or ATP in conscious rats. Naunyn-Schmiedeberg's Arch Pharmacol, 367: 473-479, 2003.

Table 1 Effects of intravesical administration of TNP-ATP (0.5μM and 5.0μM, n=6 in each), and ATP (0.1mM) in the absence and presence of TNP-ATP (0.5μM, n=6 in each) on cystometric parameters in conscious protamine-pretreated rats

	B.P.	**T.P.**	**M.P.**	**V.I.**	**M.V.**	**R.V.**	**B.C.**
TNP-ATP (0.5μM)							
before	7.3±2.3	15.9±3.7	60.0±13.3	6.57±1.60	1.07±0.27	0.06±0.02	1.13±0.28
after	8.0±1.6	17.3±4.2*	56.8±11.3	8.27±1.57**	1.34±0.25**	0.09±0.05	1.42±0.28*
TNP-ATP (5.0μM)							
before	8.1±2.3	17.0±1.5	63.8±5.9	7.39±1.07	1.20±0.17	0.08±0.02	1.27±0.19
after	11.9±4.2	22.2±2.6**	56.6±7.2	10.40±1.02***	1.69±0.18***	0.11±0.02	1.80±0.18***
ATP(0.1mM)							
before	7.5±1.7	17.4±2.1	65.8±7.3	7.47±1.45	1.21±0.24	0.07±0.01	1.28±0.24
after	8.0±1.5	16.2±2.0*	69.8±6.1	4.82±0.84**	0.77±0.14**	0.07±0.02	0.84±0.14**
ATP(0.1mM) with TNP-ATP (0.5μM)							
before	7.2±2.2	17.6±2.7	57.3±7.7	7.66±1.21	1.23±0.16	0.05±0.03	1.29±0.14
after	6.8±2.8	16.1±3.1	53.4±10.4	7.46±1.33	1.21±0.23	0.04±0.01	1.25±0.21

B.P.: Basal Pressure (cmH_2O); **T.P.**: Threshold Pressure (cmH_2O); **M.P.**: Micturition Pressure (cmH_2O); **V.I.**: Voiding Interval (minute); **M.V.**: Micturition Volume (ml); **R.V.**: Residual Volume (ml); **B.C.**: Bladder Capacity (ml)

Results are expressed as mean±standard deviation (n=6) before and after drug administration. *p<0.05, **p<0.01, ***p<0.001 (paired Student's two-tailed *t*-test)