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EVIDENCE FOR THE INVOLVEMENT OF SPINAL ENDOGENOUS ATP AND P2X RECEPTORS IN DETRUSOR OVERACTIVITY CAUSED BY ACETIC ACID, ACROLEIN OR CYCLOPHOSPHAMIDE

Hypothesis / aims of study
It has been reported that endogenous ATP from epithelial cells after bladder stretch activates purinergic receptors on submucosal afferent fibers and thus may play a major role in both volume- and pain-mediated reflexes (1). Also, it has been reported that spinal endogenous ATP pathway is activated following peripheral neurogenic and inflammatory pains and intrathecal ATP antagonists inhibit nociception (2). However, little is known about central ATP pathways in the regulation of detrusor activity. Therefore, we examined whether detrusor overactivity in acute (direct acute chemical irritation with acrolein and acetic acid) and chronic (24 hours after intraperitoneal injection of cyclophosphamide (CYP)) models could be suppressed by inhibition of P2X receptors using intrathecal administration of a non-specific P2 receptor antagonist, pyridoxal-phosphate-6-azophenyl-2′,4′,6′-disulphonic acid (PPADS) and an antagonist for the P2X1, P2X3 and P2X2/3 receptors, 2′, 3′-O-(2,4,6-trinitrophenyl)adenosine 5′-triphosphate (TNP-ATP) and activated by intrathecal specific ecto-ATPase inhibitor, ARL 67156. Also, we investigated whether ATP pathway in the spinal cord was mediated via glutamate-nitric oxide (NO) pathways.

Study design, materials and methods
Continuous cystometrograms (CMG, 0.1ml/min) were performed in female Sprague-Dawley rats under urethane anesthesia (1.2 g./kg., s.c.). Some rats received single intraperitoneal injection of CYP (200 mg./kg.) 24 hours before CMG (CYP group). In other groups, following baseline CMG recordings with saline (0.1 ml/min for 60 min), 0.25 % of acetic acid or 0.04 mg/ml acrolein were infused at the same rate (0.1 ml/min for 60 min) (acetic acid group or acrolein group). Intrathecal injection of PPADS (1 to 100 µg/rat), TNP-ATP (1 to 30 µg/rat) or ARL 67156 (50 µg/rat) were performed and CMG was recorded in the acetic acid, acrolein or CYP group as well as the control group during saline infusion. In some cases, pretreatments with MK-801, a NMDA receptor antagonist, or N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME), an NO synthase inhibitor, were administered intrathecally to investigate the involvement of glutamate-NO pathway in the spinal ATP pathway.

Results
Bladder capacity (BC) in acetic acid (0.21±0.03ml), acrolein (0.31±0.07ml) and CYP (0.14±0.03ml) groups was significantly lower than that in control (0.69±0.11ml) group, respectively. Intrathecal PPADS and TNP-ATP significantly increased the BC to the same extent in acetic acid and acrolein groups without changing in the control group. While, in the CYP group, increasing effects of PPADS on the BC was much higher than those of TNP-ATP. Intrathecal injection of ARL 67156 (50µg) significantly reduced the BC in acetic acid, acrolein and CYP groups without changing the BC in the control group. Also, pretreatment with intrathecal PPADS (100µg), MK-801 (20µg) or L-NAME (100µg) significantly suppressed the stimulatory effect of ARL 67156.(see Fig.1)

Interpretation of results
These findings suggest that spinal endogenous ATP may play a role in detrusor overactivity caused by acute chemical irritation with acetic acid and acrolein via the PPADS- and TNP-ATP-sensitive P2X receptors (perhaps the P2X\textsubscript{1} receptor subtype). After the development of inflammatory state with CYP, spinal ATP receptor subtypes related with detrusor overactivity were changed into PPADS-sensitive and TNP-ATP-insensitive P2X receptors. Also, spinal glutamate receptors and nitric oxide systems were involved in the ATP pathway for the emergence of detrusor overactivity.
Concluding message
Our results suggest that endogenous ATP in the spinal cord may stimulate afferent transmission resulting in detrusor overactivity and provide a possible basis for the use of ATP antagonists for the treatment of detrusor overactivity.

References

Fig.1 Representative tracing
CYP cystitis  PPADS (intrathecal)  \[20\text{cmH}_2\text{O}\] \[5\text{min}\]

Acetic acid (0.25%)