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INTRODUCTION & OBJECTIVE

- In humans, bladder contraction is mainly mediated by acetylcholine via muscarinic M₃ receptors (1).
- In physiopathological conditions such as aging (2), interstitial cystitis (3) or bladder instability (4), a role of ATP via P2X receptors has been reported. In addition, human urinary bladder was shown to express P2X₁ and P2X₃ receptors (5).
- A previous study in mouse isolated urinary bladder showed that a substantial component of the nerve-evoked bladder contractions is mediated by ATP through P2X₁ receptors (6).
- In anesthetized mice, the inhibitory effect of PPADS (a non selective P2 purinoceptor antagonist) on the amplitude of micturition confirmed a role of purinergic neurotransmission in the micturition reflex (7). However, the P2X receptor subtype(s) involved in this effect have not yet been investigated.
- The aim of this study was to characterize the P2X receptor subtypes mediating bladder contraction in anesthetized mice by evaluating the effects of PPADS as well as NF449 and A-317491, selective antagonists for P2X₁ and P2X₃ receptors, respectively.

MATERIALS & METHODS

- Female C57Bl6/J mice were anesthetized with urethane (1.8 g/kg, i.p.). The urethra was ligated and a jugular vein was catheterized for drug administration. The bladder was catheterized through the dome and infused with saline at room temperature to obtain a basal intravesical pressure (IVP) between 3-10 mmHg.
- α,β -MeATP (200 μ g/kg, i.v.) was administered every 20 min 2 times (basal values). Then, 10 min later, one dose of antagonist was perfused i.v. for 5 min and after a further 5 min period, α,β -MeATP was tested again. This cycle was repeated 3 times allowing us to test 4 increasing doses of one antagonist in the same animal. PPADS, NF449 and A-317491 were tested in the range 1-30 mg/kg i.v. The specificity of α,β -MeATP for P2 purinoceptors versus muscarinic receptors was determined by evaluating the effect of atropine (0.03-1 mg/kg, i.v.).
- Results were expressed as mean \pm s.e.m. of difference between IVP before and after each α,β -MeATP administration (Delta IVP, mmHg). For each antagonist, ID_{50%} (dose of antagonist required to inhibit 50% of agonist-induced bladder contraction) were calculated using linear regression.
- In each group, statistical comparisons versus basal values were performed using a one-way ANOVA with repeated measures followed by Newman-Keuls test. A p < 0.05 was accepted for statistical significance.

RESULTS

- In preliminary experiments, α,β -MeATP at 3, 10, 30, 100 and 300 μ g/kg i.v. dose-dependently increased the IVP (data not shown). Since 200 μ g/kg α,β -MeATP produced submaximal bladder contraction, this dose was chosen to test P2X receptors antagonist.
- Repeated administrations of vehicle (saline) did not elicit any appreciable change in the bladder contractions induced by α,β -MeATP except for the last administration which induced a weak but significant increase of IVP with respect to basal values (Figures 1 and 2).
- Basal values of α,β -MeATP-induced bladder contractions were not statistically different between control and treated-groups (p>0.05 by Student t test).
- PPADS (n=8) and NF449 (n=8) dose-dependently inhibited α,β -MeATP-induced bladder contractions (Figure 2). These effects were statistically significant starting from 1 and 3 mg/kg i.v. for PPADS and NF449, respectively. At the maximum dose tested, PPADS and NF449 almost totally inhibited α,β -MeATP effects, by -92 \pm 2 and -88 \pm 5 %, respectively. Neither A-317491 (n=6) nor atropine (n=4) modified α,β -MeATP-induced bladder contractions (Figure 2).

Figure 1: Typical recordings of the effects of vehicle (above) and NF449 (below) on α,β -MeATP-induced increase of IVP in anesthetized female mice.

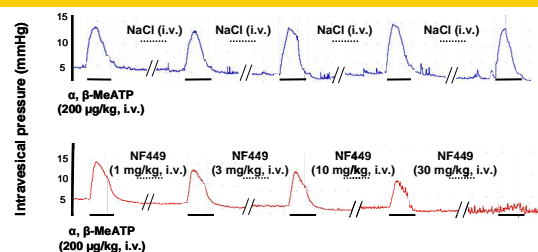
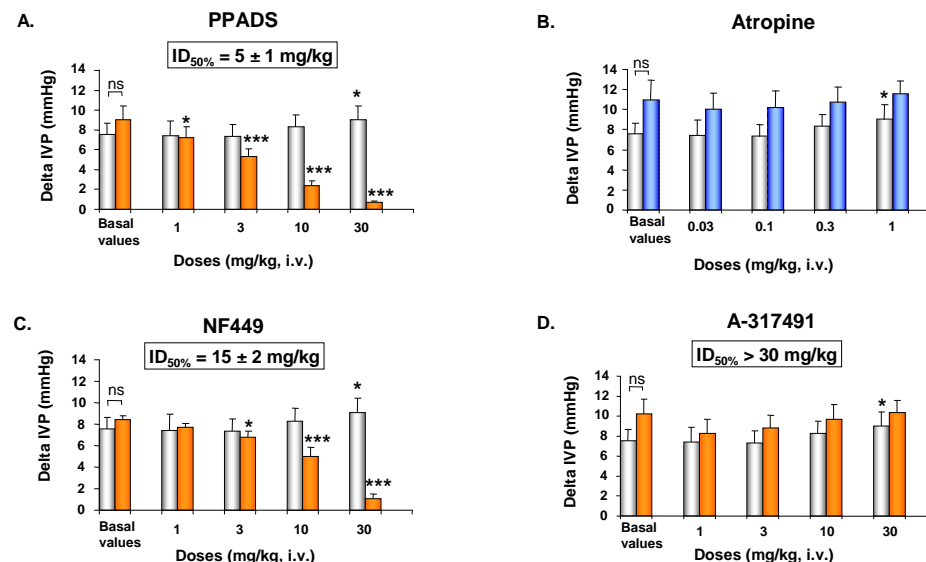


Figure 2: Effects of vehicle (gray bars, n=8), atropine or purinergic antagonists (color bars) on α,β -MeATP (200 μ g/kg, i.v.)-induced increase of IVP in anesthetized female mice. A: PPADS (n=8), B: atropine (n=4), C: NF449 (n=8), D: A-317491 (n=6). *p<0.05, ***p<0.001 vs basal values by one-way ANOVA with repeated measures followed by Newman-Keuls test. ID_{50%}: dose of antagonist required to inhibit 50% of agonist-induced bladder contraction.



CONCLUSIONS

- In anesthetized mice, α,β -MeATP induced an increase of IVP. This effect was inhibited by PPADS but not atropine suggesting that α,β -MeATP mediates this effect by activating P2X receptors.
- NF449, a P2X₁ receptor antagonist having very high affinity for rat (8) and human (9) P2X₁ receptor, dose-dependently inhibited α,β -MeATP-induced effects. Thus, the effect of α,β -MeATP on this experimental model is mediated by P2X₁ receptors in analogy with results reported in the mouse isolated urinary bladder (6).
- Up to 30 mg/kg i.v., A-317491 did not inhibit the α,β -MeATP-induced effects. This suggests that P2X₃ receptors are not involved in α,β -MeATP-induced bladder contraction in mice. In addition, this result indicates the strong selectivity of A-317491 for P2X₃ versus P2X₁ receptors.
- In conclusion, this experimental model represents a reliable tool for *in vivo* screening of new P2X₁ receptor antagonists for the treatment of overactive bladder in humans.

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