PURINERGIC CONTRACTILE EFFECTS MEDIATED BY CHOLINERGIC TRANSMISSION IN THE RAT URINARY BLADDER

Hypothesis / aims of study
The investigation of the individual roles of adenosine 5′-triphosphate (ATP) in the urinary bladder usually focus on the effects of ATP as a non-cholinergic (and usually also non-adrenergic) transmitter. While there are several studies indicating a link between purinergic and cholinergic transmission, the results in terms of functional effects in the urinary bladder differ between studies. In this preliminary study, direct correlations between purinergic and cholinergic signalling were investigated by employing a novel in situ half-urinary bladder model.

Study design, materials and methods
In the current study, an in situ half bladder experimental set up was employed and compared with traditional, well-established in vitro organ bath studies. The in situ experiments were conducted in the same manner as has previously been described (1). In short, male Sprague Dawley rats (340-460 g) were anesthetized with pentobarbital (30 mg/kg i.p.) and medetomidine (10 µg/kg i.p.). Cannulas were inserted into the femoral vein and artery, for drug administration and blood pressure recording, respectively. The urinary bladder was prepared via a midline cut and ligatures were fixated at each side of the bladder, which thereafter completely separated into two halves all the way to the urethra. Functional contractions were measured from one of the bladder halves, whereas the contralateral side was left untouched. In some experiments, the contralateral pelvic nerve was cut in order to abolish afferent signalling. The in vitro experiments were conducted in a similar fashion, where full thickness bladder strip preparations were cut out from the urinary bladder and mounted in organ baths filled with Krebs solution as described previously (2). The computed sample size was n=6 with a power of 80%. Statistics were calculated using 1-way or 2-way ANOVA with Tukey's HSD post hoc test. Values are presented as mean±SEM.

Active substances used in these experiments were the muscarinic agonist methacholine, the muscarinic antagonist atropine and the purinergic agonist ATP.

Results
In vitro, methacholine evoked larger maximal contractile responses than did ATP (33.0±2.1 and 7.5±1.6 mN, for methacholine (10-4 M) and ATP (5×10-3 M), respectively n=8 in each group; p<0.01), which is in concordance with previous studies. In situ, however, the two substances gave a similar maximal contraction (2.4±0.8 mN (2 µg/kg i.v.; n=8) and 3.8±1.1 mN (10 µg/kg i.v.; n=8) for methacholine and ATP at similar molar concentrations ≈0.015 µmol/kg). Cutting the pelvic nerve did not lower neither the ATP- nor the methacholine-evoked responses. The responses to ATP (10 µg/kg i.v.) and methacholine (5 µg/kg i.v.; n=4), were 88±5% and 98±2%, respectively, of the responses before cutting the nerve. Interestingly, administration of atropine (1 mg/kg i.v.) did not only abolish the methacholine-evoked contraction, but did also markedly reduce the contractile responses to ATP (at 10 µg/kg from 4.5±1.0 to 1.2±0.2 mN before and after atropine, respectively; n=4, p<0.01).

Interpretation of results
The results from this preliminary study suggest that the link between purinergic and cholinergic signalling may appear differently when studied in vitro and in vivo/in situ. Other studies have seen various effects of atropine on ATP-induced contractions (3), but the impact previously reported is smaller than what is currently observed in situ. Thus, we hypothesised that ATP likely plays an important afferent role in vivo/in situ. However, cutting the contralateral pelvic nerve (in situ-model), and thereby disabling a large part of the afferent signalling, did not affect the contractions to the agonists. On the other hand, atropine markedly reduced the contractile response to ATP, suggesting a direct involvement of acetylcholine.

Concluding message
Taken together, the current preliminary results imply that ATP is involved in the contraction of the urinary bladder through the release of acetylcholine on a level likely to be different than on afferent nerve-endings in the urinary bladder. This link is suggested to be exerted at a higher (CNS) level, but an effect on efferent nerves in the bladder wall can not be excluded in view of in vitro reports of a certain degree of atropine-sensitivity.

References

Disclosures
Funding: Wilhelm och Martina Lundgrens Vetenskapsfond, Rådman och Fru Ernst Collianders Stiftelse, Kungliga och Hvitfeldtska stiftelsen and Vetenskapsrådet. Clinical Trial: No Subjects: ANIMAL Species: Rat Ethics Committee: Local ethics committee at the University of Gothenburg