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De Bock F[1], De Wachter S[1], Wyndaele J J[1]
1. *University of Antwerp, Urology*

THE EFFECT OF INTRAVESICAL ELECTRICAL STIMULATION UPON THE RAT WHOLE BLADDER ACTIVITY: IN VIVO AFTER NERVE TRANSSECTIONS AND IN VITRO AFTER TREATMENT WITH ATROPINE, ALPHA,BETA-METHYLATP AND TETRODOTOXIN.

Hypothesis / aims of study

Intravesical electrical stimulation (IVES) has been used to increase bladder sensations and to improve bladder emptying. The exact working mechanism of IVES is still subject of discussion. Previous studies showed that IVES is mainly nerve mediated, but even after all nerves were cut, IVES could induce a detrusor contraction. In this study we tried to further explore the effect of IVES in decentralized bladders and to study the importance of sensory receptors in the bladder wall for a response upon IVES.

Study design, materials and methods

16 female Sprague-Dawley rats were anesthetized with urethane. A single lumen catheter was introduced transurethrally and used for bladder filling and emptying, for electrical stimulation (300s, square wave, 10Hz, 20ms pulse duration and 6mA amplitude) with platinum-iridium wire (anode) and for recording bladder pressure as response parameter to IVES. The bladder was filled with saline up to 50% of the threshold bladder volume. Bladder pressure development with filling and with IVES was measured *in vivo* before (baseline) and after the bladder nerves were consecutively sectioned at different levels. L6 roots (bilateral), pelvic nerve (bilateral) and major pelvic ganglion (MPG) with surrounding nerves. Afterwards the whole bladder was removed and mounted in a tissue bath. IVES was performed *in vitro* in all 16 bladders without treatment (control). For 6 bladders, Tetrodotoxin (TTX; 1 µM) was added to the tissue bath. Six other bladders were first treated with atropine (1 µM) and after washout, atropine (1µM) and α,β-methylATP (5 x 10µM, desensitisation of P2X purinoceptors) were added together. Finally 4 bladders were treated first with α,β-methylATP (5 x 10 µM) and after washout with α,β-methylATP (5 x 10 µM) and atropine (1 µM). After each treatment IVES was carried out.

Results

Transsection of the roots, the pelvic nerves and MPG resulted in a maximal pressure rise (maxΔP) of respectively 42 ± 9%, 33 ± 10% and 23 ± 6% of the baseline (maxΔP in intact *in vivo* bladder) due to IVES. These maxΔP were significantly lower than maxΔP of the baseline (resp. p-values are 0.01, 0.00 and 0.00). The IVES evoked pressure rise after transsection of the MPG and surrounding nerves was also significantly lower than maxΔP after roots transsection (p=0.00 and statistical power is 0.9) and after pelvic nerve transsection (p=0.04 and statistical power is 0.7) (Figure 1a.).

The *in vitro* control maxΔP was 45 ± 8% of the *in vivo* baseline. After treatment with TTX, the pressure rise as response upon IVES was abolished.

Treatment with only atropine did not change the maxΔP (91 ± 2% of the *in vitro* control) (Figure 1b.). Treatment with only α,β-methylATP reduced the intravesical pressure rise upon IVES to 20 ± 9% of the *in vitro* control. Treatment with both α,β-methylATP and atropine abolished IVES induced pressure rise. An IVES induced pressure rise of 68 ± 14% of the *in vitro* control was recorded after α,β-methylATP and atropine were washed out in 4 bladders (Figure 1c.). The slope of the pressure decrease, after maxP was reached, was significantly steeper when atropine was added than the slope of the control (p=0.01). The statistical power of the analyses ranged between 0.7 and 1 with a mean of 0.9.

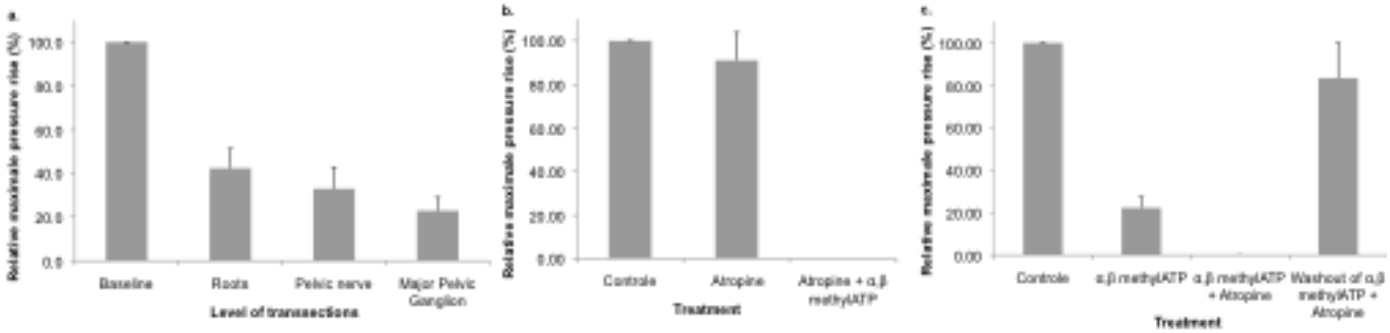

Figure 1.: a. Relative maximal pressure rise *in vivo* in 16 rats; b. Relative maximal pressure rise *in vivo* in 6 rats; c. Relative maximal pressure rise *in vivo* in 4 rats. (The error bars indicate the standard error of the mean)

Interpretation of results

After transsection of the MPG (including the hypogastric nerve which passes through the MPG), the IVES induced pressure rise decreased significantly, but was still present. This decrease suggests the presence of a reflex pathway, before transsection of the MPG and through the MPG, that can electrically be activated and thus enhance the strength of the IVES evoked detrusor contraction. It cannot be concluded that the course of the pathway is purely peripherally from the spinal cord, since the bladder was still connected to the spinal cord through the hypogastric nerves when MPG and surrounding nerves are still intact. However, a local reflex pathway through only the MPG seems less likely because as for now, no clear evidence (physiological and anatomical) has been found to proof possible afferent-efferent interaction in the MPG (1). It also has been shown that inputs of the hypogastric and pelvic nerve can activate the same MPG neurones, which suggest a connection between hypogastric preganglionic nerves and pelvic postganglionic nerves that innervate the bladder (2). This could mean that the hypogastric nerves can influence the bladder contraction by activating these common neurones. The IVES evoked contraction after MPG transsection can be the result of stimulation of the detrusor muscles, the efferent or afferent nerve endings, the urothelium or the interstitial cells.

In vitro, we showed that in this study the bladder response upon IVES is not the result of direct activation of the detrusor muscles, because contraction was abolished after treatment with TTX. IVES causes a release of cholinergic and purinergic neurotransmitters which mediate the detrusor contraction. As found in tissue baths, IVES evoked contraction can also be divided in two components

(3). One of them, the tonic component (the duration of the pressure rise), can be regulated by muscarinic receptors and be blocked by atropine. The phasic component (the height of the pressure rise) can be regulated by purinergic receptors and be blocked by desensitisation with α,β-methylATP.

Concluding message

Our results suggest the presence of a reflex pathway possibly within the MPG but more likely from the spinal cord through the hypogastric nerve and passing the MPG. This reflex pathway can be activated electrically. The IVES induced contraction after transsection of MPG is certainly not a result of direct bladder muscle stimulation.

The *in vitro* experiments revealed that a whole rat bladder reacts upon IVES in a similar way as bladder detrusor strips on electric field stimulation. The contraction evoked by IVES can also be divided in two parts, a cholinergic and a purinergic part. The purinergic part is mainly responsible for the contraction strength. Further research is needed to reveal which bladder wall structures are involved in mediating IVES induced detrusor contraction.

References

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