# THE INFLUENCE OF AMBROXOL AND CAPSAICIN ON THE ISOLATED RABBIT BLADDER WALL

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Abstract: Unmyelinated C-fibers endings lye beneath the epithelial layer and release neuropeptides which regulate baseline transepithelial potential difference (PD) and changes of transepithelial potential difference during mechanical stimulation (dPD). Ambroxol can suppress reflexes such as the cough or the corneal reflex which are connected to stimulation of C fibre endings. The study aimed to estimate the influence of ambroxol (ABX) and capsaicin (CAPSA) on PD and dPD in isolated rabbit bladder. The experiments were carried out on 26 bladder specimens of. 2 cm<sup>2</sup> surface area each, obtained from 13 rabbits. Ussing apparatus was used. Procedure used for stimulation of sensory receptor involved directing stream onto epithelium. A stimulus lasted 30s, ejecting the 2.5 ml fluid. Amiloride and bumetanide were used to estimate of ionic currents. Each significant reaction was repeated at least 10 times on various specimens. PD ranged between 5 and 10 mV in different experimental conditions. Mechanical stimulation of isolated bladder wall caused increasing dPD about 2 mV defined as hyperpolarization. Application of ABX to the stimulation fluid decreased the hyperpolarization in comparison with control stimulation under conditions of inhibited chloride ion transport. Application of CAPSA to the stimulation fluid decreased the hyperpolarization in comparison with control stimulation under conditions of inhibited sodium ion transport. ABX and CAPSA had no influence on PD after mechanical stimulation. ABX as well as CAPSA influence on transpithelial ion transport pathways in bladder epithelium is dependent on sensory stimulation. ABX can be suspected to suppress bladder contractions.

Keywords: C-fibers, epithelial nerve endings, ambroxol, capsaicin, overactive bladder

Incontinence and overactive bladder affect more than 17 million Americans. Overactive bladder leads to distressing symptoms such as urgency, frequency and urine incontinence. Hypersensitive disorder of the lower urinary tract and interstitial cystitis, a syndrome characterized by motor and sensory bladder dysfunction, represent a diagnostic and therapeutic challenge even to highly skilled urologists [1]. Capsaicin inhibits rhythmic bladder contractions, suggesting that activity in sensory fibers (C and A delta), which are sensitive to the action of this drug, is initiated by bladder filling [2]. Capsaicin-sensitive C-fibers drive a spinal segmental reflex pathway, causing probably neurogenic detrusor overactivity. Unmyelinated C-fibers are located beneath the epithelium.

There is great hope for future research on voiding dysfunction and urinary incontinence

through a focus on afferent nerve intervention for preventing overactive bladder. Ambroxol can suppress reflexes such as the cough or the corneal reflex which are connected to stimulation of C fibre endings in airways. Preclinical study shows that local anaesthetic properties of ambroxol hydrochloride may have beneficial clinical implications [3]. There is a possibility that ambroxol can suppress urinary bladder contractions and it will be a great alternative for intravesically management on overactive bladder.

The aim of this study was to estimate of the influence of ambroxol (ABX), and capsaicin (CAPSA) on transepithelial electrical potential difference (PD) and changes of transepithelial potential difference during mechanical stimulation (dPD) in isolated rabbit bladder.

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#### EXPERIMENTAL

Animals: The experiments were performed on 26 specimens of isolated bladder wall from 13 rabbits (2 specimens from animal) from the university animal breeding stock. For the experiment, rabbits (with free access to water and food prior to study) of both sexes and weighting between 3.5-4.0 kg were used. Rabbits were killed at 9 a.m with CO<sub>2</sub> asphyxiation. After incision of abdominal wall, the bladder was gently excised and cut longitudinally into two pieces of about 2.0 cm<sup>2</sup> surface area each. The experiments were approved by Bydgoszcz Universities' Committee for Ethical Animal Experiments.

Electrical potential measurement: The solutions, concentrations are given in mM in parentheses, used in the experiments were: RH - Ringer solution (Na 147.2, K 0.4, Ca 4.4, Cl 156.6, Hepes 10.0) buffered to pH 7.4; AMI (amiloride is an inhibitor of sodium ion transport pathway) amiloride (0.1) dissolved in and diluted with Ringer solution; BUME (bumetanide is an inhibitor of chloride ion transport pathway) – bumetanide (0.1) dissolved in DMSO (dimethyl sulfoxide, final concentration 1%) and diluted with Ringer solution; AB amiloride and bumetanide (both 0,1) dissolved in DMSO and diluted with Ringer solution. ABX ambroxol (0.1) dissolved in and diluted with Ringer solution; CAPSA - capsaicin (0.1) dissolved in DMSO (final concentration 1%) and TWEEN 80, and diluted with Ringer solution; all supplied by Sigma-Aldrich Ltd., Poland.

After about 60 min of incubation in aerated Ringer solution, AMI solution or BUME solution specimens were mounted between elastic gaskets to avoid the edge damage in the Ussing apparatus filled with bathing fluid. To minimize bulging, specimens were put on the filter paper support. The Ussing apparatus was equipped with the nozzle (approximately 1.2 mm in diameter and set 12 mm away from tissue) connected to a peristaltic pump. The jet flux of stimulation fluid from the nozzle washed the mucosal surface of the tissue. The standard stimulus was 11-12 jets, with the total volume of 2,5 mL, and lasted about 30 s. The area of the tissue under study was 1.0 cm<sup>2</sup>. The volume of Ussing half chamber was 10.0 mL. The Ussing chambers were permanently opened, with the aim to avoid any transepithelial pressure gradients.

After an equilibration period of 20 min, electrical parameters of the isolated tissue were determined. The experimental procedures with different bathing and stimulation fluids were applied. Each type of experiment was repeated more than 10 times on the specimens from different animals. The series of stimulation with consecutive addition of bumetanide, amiloride and both (0.1 mM) to stimulation fluid were performed at the end of experiments.

The chamber halves of the Ussing apparatus were connected with two pairs of agar bridges to Ag/AgCl electrodes which were linked with the EVC 4000 voltage/current clamp amplifier (WPI, USA). The EVC apparatus was connected to the data acquisition system MP 100 (BIOPAC Systems, Inc, California) and subsequently to the computer. First pair of electrodes was used for measurement of transepithelial potential difference (PD), and second for passing of current of  $\pm 10 \ \mu$ A through the tissue. Tissue resistance was then calculated according to Ohm's law.

**Statistical analysis:** All values are expressed as mean  $\pm$  S.D. t-Student test was used to determine the statistical significance of differences between means. A probability value of 0.05 or less was considered to be statistically significant.

## RESULTS

Electrophysiological parameters of isolated rabbit bladder were summarized. Transepithelial electrical potential difference (PD) ranged between 1 and 8 mV under different experimental conditions. Mechanical stimulation of isolated bladder wall caused increasing transepithelial electrical potential difference (dPD) of about 2 mV defined as hyperpolarization. It was shown that application of ambroxol and capsaicin to the stimulation fluid decrease dPD compared to control under uninhibited conditions (RH). No influence on PD was observed after capsaicin and ambroxol application to the stimulation fluid under uninhibited conditions (RH) compared to control (Table 1, RH, Figure 1).

To evaluate which transpithelial ion transport pathways was influenced after application of ambroxol and capsaicin under baseline (PD) and stimulated (dPD) conditions sodium ion transport inhibitor – amiloride or chloride ion transport inhibitor – bumetanide were applied to all experimental fluids.

It has been observed that application of ambroxol decreased dPD under inhibited chloride ion transport and capsaicin decreased dPD under inhibited sodium ion transport (Table 1, AMI, BUME, Figure 1).

### DISCUSSION

The voiding reflex requires afferent output from the bladder to the central nervous system and



Figure 1. The influence of capsaicin (A, B, C) and ambroxol (D, E, F) on PD and dPD in isolated rabbit bladder wall.

efferent nerve input to the bladder from the spinal cord. Afferent bladder nerves are critical for sending signals of fullness and discomfort to the brain. The bladder afferent pathways consist of small myelinated A-delta and unmyelinated C-fibers. A-delta fibers transmit signals mainly from mechanoreceptors that detect bladder fullness or wall tension, while the C-fibers mainly detect noxious signals and initiate painful sensations. Bladder infection or irritative condition is the situation when C-fibers conduct signal to the central nervous system. C-fiber afferents have reflex function also to facilitate or trigger voiding, which can be considered a defense mechanism to eliminate irritants or bacteria [4,5]. Interruption of the spinal cord pathways produces considerable reorganization of the micturition reflex

and C-fiber bladder afferents become mechanoreceptive and initiate voiding reflex [6].

Capsaicin, as an irritant drug, is used in *in vitro* and *in vivo* studies for defining contribution of C-fiber in reactions after mechanical stimulation [5]. Capsaicin interacts with the specific recognition site, vanilloid receptor subtype I [7]. Clinical application of intravesical capsaicin for voiding dysfunction was first reported by Maggi et al. in patients with bladder hypersensitivity [8]. In patients with multiple sclerosis or spinal cord injury who had overactive bladder 1 to 2 mM capsaicin intravesically decreased significantly bladder hyperactivity and/or the number of urinary incontinence episodes. However, capsaicin causes initial stimulation of unmyelinated C-fibers, resulting in severe discom-

		Control	Capsaicin (CAPSA)
RH	PD	$-2,15 \pm 1,05$	$-1,84 \pm 0,86$
(n=15)	dPD	$-0,38 \pm 0,18$	$-0,17^* \pm 0,17$
AMI	PD	$-1,86 \pm 1,23$	$-1,27 \pm 0,96$
(n=8)	dPD	$-0,28 \pm 0,29$	$-0.04^* \pm 0.19$
BUME	PD	$-2,27 \pm 2,4$	$-2,0 \pm 1,88$
(n=16)	dPD	$-1,06 \pm 0,9$	$-0.95 \pm 1.01$
		Control	Ambroxol (ABX)
RH	PD	$-2,15 \pm 1,07$	$-2,04 \pm 0,9$
(n=24)	dPD	$-0,58 \pm 0,32$	$-0,35^* \pm 0,32$
AMI	PD	$-1,5 \pm 0,63$	$-1,44 \pm 0,58$
(n=8)	dPD	$-1,15 \pm 0,23$	$-0,15 \pm 0,2$
BUME	PD	$-3,32 \pm 2,9$	-3,14 ± 2,85
(n=25)	dPD	$-0,69 \pm 0,68$	$-0,33^* \pm 0,25$

Table 1. The influence of ambroxol and capsaicin on PD and dPD in isolated rabbit bladder wall.

The mean  $\pm$  S.D. value are given; n – number of experiments given in parenthesis, PD – baseline transepithelial electrical potential difference (mV), dPD – the difference between maximum value after stimulation and baseline PD value (mV). Experimental conditions: in the control group; incubation, bathing and stimulation fluids; RH (Ringer solution), AMI (amiloride) and BUME (bumetanide); in the Capsaicin group: capsaicin was added to stimulation fluids; in the Ambroxol group: ambroxol was added to stimulation fluids.

\* – statistically significant difference with comparison to control group (p < 0.05).

fort or pain, with release of the neurotransmitters substance P and/or neurokinin A in the bladder. In a recent capsaicin study of detrusor hyperreflexia 44% of patients had satisfactory continence, 36% were improved and treatment failed in only 20% [1].

It was shown that the transepithelial electrical potential difference of the tissue was hyperpolarized transiently after mechanical stimulation by means of gentle rinsing of its mucosal side. This hyperpolarization was greatly influenced by the addition of serotonin and/or ambroxol to the stimulation (rinsing) fluid. By means of selective blocking of ion transport transepithelial pathways with amiloride and/or bumetanide, the effects of serotonin and/or ambroxol on the hyperpolarization after mechanical stimulation could be explained as changes of sodium ion currents. The importance of stimulated ionic currents for airway clearance and for the efficacy of drugs acting on airways was postulated [9].

A stimulation of afferent neuronal endings in such epithelial organs as airways, colon and frog skin produces reversible hyperpolarization, usually explained as caused by neuropeptides released from sensory endings [10]. The ambroxol influence on smooth muscle and epithelial cells with respect to ion transport may be dependent on NANC neuropeptides action [11]. The effect of ambroxol on the elements of neurohormonal intramural regulation systems of secretory epithelia additionally explains and justifies the potentcial therapeutic value of the drug in the lower urinary tract. It was, for example, shown that ambroxol can influence the sodium transpithelial transport pathway which is partly responsible for the hyperpolarization during mechanical stimulation of the mucosal surface of tracheal wall [12]. Kosik-Bogacka & Tyrakowski demonstrated, based on the experiments carried out frog skin model, that ambroxol can modify processes of ion transport related to activation of sensory receptors. In particular, ambroxol can influence the stimulated transpithelial sodium ion transport in frog skin [11]. In this work, authors observed ambroxol influence on hyperpolarization under inhibited chloride ion transport pathways.

It was the first proof that ambroxol influences the bladder wall ionic currents. It should be examined if the ambroxol influences the bladder wall under the inflammation conditions. There is a possibility that ambroxol can suppress urinary bladder contractions and it will be a great alternative for intravesical management of overactive bladder.

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