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# ROLE OF TRANSMEMBRANE CALCIUM CURRENT IN ANGIOTENSIN II-MEDIATED CONTRACTION OF DETRUSOR ORGAN STRIPS FROM RAT URINARY BLADDER

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**Summary**

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The changes of contractile activity of smooth muscle strips from urinary bladder of Wistar rats induced by angiotensin II were examined. The preparations were subjected to the influence of medium containing high calcium concentration to establish the participation of extracellular calcium in smooth muscle contraction. The role of membrane calcium channels in angiotensin II-induced contraction was investigated by application of selective (1-octanol) and nonselective (nicardipine) calcium channel blockers. Angiotensin II (1 μM) and $CaCl_2$ (10 mM) provoked contractile response with maximum amplitudes of 1.57±0.5 g and 1.5±0.6 g, respectively. Both types of calcium channel antagonists inhibited the contractile response of the urinary bladder detrusor. Our results showed a greater significance of calcium-induced calcium release than inositol-triphosphate-induced calcium release in tonic contraction of detrusor muscle strips.

**Key words**: 1-octanol, angiotensin II, nicardipine, organ bath, urinary bladder

## INTRODUCTION

Angiotensin II is described as being primarily a vasoconstrictor peptide. A lot of recent studies have shown that angiotensin II has also cytokine and growth factor-like properties (Kim & Iwao, 2000; Touyz & Berry, 2002). It affects non-vascular tissues as well. The information on the effect of angiotensin II on non-cardiovascular organ smooth muscles is insufficient (Touyz & Berry, 2002). The effects of angiotensin II on the urinary bladder are of particular interest with regard to the pathogenesis and treatment of micturition disorders. The physiological role of angiotensin II on the function of the urinary bladder and the exact transductional mechanisms mediating its effects have not been fully elucidated.

There is evidence that *in vitro,* angiotensin II induces contraction of rat urinary bladder smooth muscle strips. This contraction is dose-dependent and is inhibited by losartan – a selective angiotensin II type 1 receptor antagonist (Chiu *et al*., 1994). These data suggest a possible role of angiotensin II in micturition (Timmermans *et al*., 1993). Andersson & Arner (2004) put forward the hypothesis that

angiotensin II participates in urinary bladder neurotransmission as a neuromodulator (Andersson & Arner, 2004). In experimentally induced urethra obstruction and increased basal muscle strip length, local overproduction of angiotensin II is observed, which on its part influences the length of muscle fibres through a direct effect on muscle tone (Cheng *et al*., 1999).

Angiotensin II is known to exert its physiological effects through binding to membrane angiotensin I and II receptors (De Gasparo & Levens, 1998; Touyz & Berry, 2002). Angiotensin I receptors are known to activate phospholipase C, dihydropyridine-sensitive $Ca^{2+}$-channels and to inhibit adenylyl cyclase thus reducing intracellular cyclic adenosine monophosphate levels (Chiu *et al*., 1994). There are contradictory data concerning the effect of angiotensin II on T-type voltage-dependent $Ca^{2+}$-channels (VDCC).

The increase of intracellular $Ca^{2+}$ is undoubtedly a key process in the activation of detrusor contraction. It is still debatable whether this increase is due to extracellular influx and/or to release from intracellular stores and the importance of each particular mechanism in different animal species is discussed (Kajioka *et al*., 2002).

The aim of the present study was to examine the role of transmembrane calcium current in angiotensin II-mediated contraction of detrusor organ strips from rat urinary bladder.

## MATERIALS AND METHODS

Six male and 7 female Wistar rats weighing 200–250 g were used. Experimental animals were anaesthetized with ketamine hydrochloride (Calypsol, Gedeon Richter, Hungary) at a dose of 10 mg/kg intraperitoneally and exsanguinated. The experiment was carried out in accordance with the national regulations and European Directive 86/609/EEC of Nov 24/1986 concerning the protection of animals used for scientific and experimental purposes.

Abdominal cavity was opened and the urinary bladder was dissected out and immediately placed in cold Krebs solution (3°C) (containing in mM: NaCl – 118.0, KCl – 4.75, $NaHCO_3$ – 25.0, $MgSO_4$ – 1.2, $CaCl_2$ – 2.0, $KH_2PO_4$ – 1.2 and glucose – 11.0). Organ strips 8 mm long were cut out and mounted in organ baths TSZ-04/01, containing 5 ml Krebs solution with constant temperature (37°C), pH 7.4, continuously bubbled with Carbogen (95% $O_2$ and 5% $CO_2$).

### *Recording of mechanical activity*

The two ends of each organ strip were tied with silk thread. The distal end was connected to the organ holder, the proximal end was stretched, and was attached to a mechanoelectric transducer FSG-01 (Experimetria, Ltd., Hungary) via a hook. Mechanical activity was digitized and recorded using ISOSYS 1.0 computer programme (IsoSys 1.0., 2003). The conversion of data into digital format for later analysis was performed with KORELIA – IZOSYS programme (Yankov, 2007), and the analysis and graphic processing – with Korelia – Dynamics (Yankov, 1998 a, b). Longitudinal preparations were subjected to an initial tension (preload) of 1 g followed by three periods of equilibration: 15 min, 45 min and 15 min. Two washouts with Krebs solution were performed between equilibration periods. All preparations displayed rhythmic spontaneous contractions.

### *Experimental protocols*

After a period of equilibration, angiotensin II was administered in supramaxi-

mum concentration of 1 μM for the induction of non-cumulative contractions. The role of $Ca^{2+}$ in angiotensin II-mediated contraction was tested via addition of 10 mM $CaCl_2$ before angiotensin II and via addition of two transmembrane $Ca^{2+}$- influx inhibitors (10 μM 1-octanol and 10 μM nicardipine) administered immediately after angiotensin II.

*Chemicals and drugs*

Angiotensin II, 1-octanol (a selective T-type VDCC blocker) and $CaCl_2$ were solubilized in bidistilled water and nicardipine (a non-selective T (L) calcium channel antagonist) was solubilized in dimethyl sulfoxide. All reagents, including the chemicals for the preparation of Krebs solution were purchased from Sigma-Aldrich.

*Statistical analysis*

Experimental data were expressed as means ± SD for 2n, the number of preparations (n = the number of animals) and analysed with the Student's *t*-test at a level of significance $p<0.05$ using statistical software (Statistica 6.1, StatSoft Inc.)

## RESULTS

After an equilibration period, the preload was decreased to 0.48±0.13 g. On the background of this baseline isolated strips from urinary bladder detrusor displayed spontaneous activity with a maximum amplitude of 1.14±0.13 g (Fig. 1). Angiotensin II at concentration 1 μM induced tonic contraction with a maximum amplitude of 1.57±0.5 g. The increase of extracellular $Ca^{2+}$ with 10 mM $CaCl_2$ resulted in tonic contraction with a maximum amplitude of 1.5±0.6 g followed by phasic contractions with gradually decreasing amplitude. There were no statistically significant differences between maximum amplitudes of contractions provo-

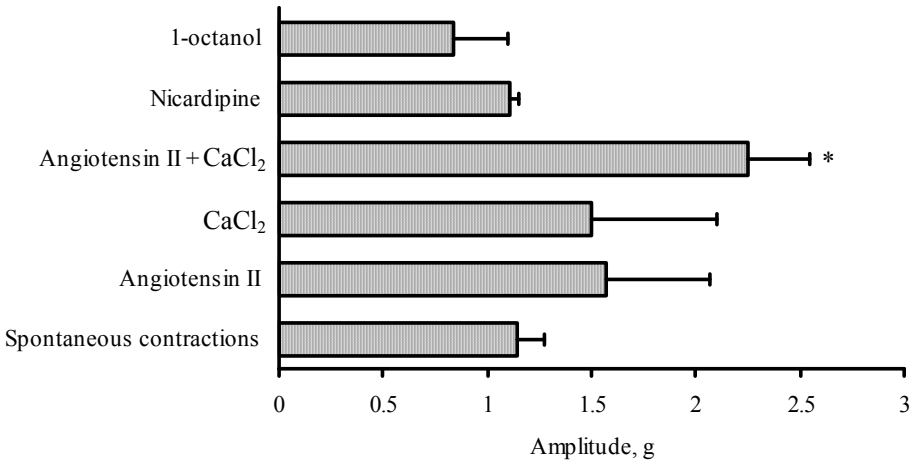


**Fig. 1.** Maximum amplitude of contraction (g) of detrusor organ strips from rat urinary bladder following application of angiotensin II, $CaCl_2$, angiotensin II in the presence of increased extracellular $Ca^{2+}$ after addition of $CaCl_2$ and VDCC blockade with either a non-selective antagonist (nicardipine) or a selective T-type Ca-channel antagonist (1-octanol). Values are presented as means ± SD (2n=26); $P<0.05$ vs spontaneous contractions.

ked by angiotensin II and $CaCl_2$ (Fig. 1).

The addition of the same dose of angiotensin II in the presence of 10 mM extracellular $Ca^{2+}$ increased significantly ($P<0.05$) the maximum amplitude of contraction to 2.25±0.3 g (Fig.1).

The non-selective Ca-channel blocker nicardipine (10 μM) added immediately after angiotensin II (1 μM), blocked angiotensin–induced tonic contraction. Phasic contractions with a maximum amplitude of 1.11±0.04 g, not statistically different from the spontaneous activity, were registered (Fig. 1).

In the presence of angiotensin II (1 μM), the selective T-type Ca-channel antagonist 1-octanol (10 μM) demonstrated a similar effect i.e. lack of tonic contraction and registration of phasic contractions with a maximum amplitude of 0.84±0.26 g. The amplitude of phasic contractions reached after administration of l-octanol changed statistically insignificantly vs both spontaneous activity and nicardipine-induced phasic contraction amplitudes (Fig. 1).

## DISCUSSION

It is well established that the detrusor smooth muscle from urinary bladder displays spontaneous rhythmic activity *in vivo* and *in vitro*. These spontaneous contractions vary in amplitude, as well as in frequency, depending on the investigated animal species. There are conflicting data in the literature regarding the role played by different types of VDCC in the generation of spontaneous contractile activity (Kushida *et al*., 2001; Andersson & Arner, 2004). According to Fry *et al*. (1998) the contractile activity of smooth muscle strips is dependent on the stretch of the preparation as well. When the length of the preparation is above 20% of its resting length, the influx of $Ca^{2+}$ through $Ca^{2+}$-channels is sufficient to increase intracellular $Ca^{2+}$ and to induce membrane depolarisation. This results in opening of L-type $Ca^{2+}$-channels, pronounced increase of $Ca^{2+}$ influx leading to contraction. *In vitro* experiments on guinea pig detrusor muscle strips performed by Hashitani *et al*. (2004) showed that the calcium channel blocker nifedipine at a concentration of 1 μM completely blocked the spontaneous contractile activity in 40% of the preparations (Hashitani *et al.,* 2004). The same effect was observed with the rest of the preparations but when nifedipine concentration was increased from 10 to 30 μM. The authors speculate that L-type $Ca^{2+}$-channels play a pivotal role in spontaneous activity. Similar *in vitro* experiments on guinea pig detrusor demonstrated that the blockade of T-type $Ca^{2+}$-channels influenced spontaneous contractile activity (Chow *et al*., 2003). In the course of our experiments, the administration of nicardipine, a blocker of both types of channels, did not induce any significant changes in the spontaneous activity of the detrusor muscle. The administration of 1-octanol, a selective blocker of T-type VDCC, exhibited a tendency to decrease in the amplitude of spontaneous contractions with no statistically significant difference. The lack of pronounced effect resulting from the blockade of the two types of VDCC channels gives us reasons to discuss the role of stretch-activated $Ca^{2+}$ channels in the manifestation of spontaneous contractile activity.

The existence of c-Jun N-terminal kinase (JNK) which is activated by mechanical stretch and is totally dependent on extracellular $Ca^{2+}$, but is not inhibited by calcium channel blockers, has been established in detrusor smooth muscle cells (Kushida *et al*., 2001). The observa-

tion of tonic contractions following the increase of extracellular $Ca^{2+}$ with $CaCl_2$ could be explained by the additional activation of calcium-dependent stretch-activated JNK.

Tonic contractions induced by the administration of angiotensin II only at supramaximum concentrations confirmed data from literature concerning its effect on urinary bladder contractile activity. The increased amplitude of contraction following the administration of angiotensin II at the same concentration in the presence of increased extracellular $Ca^{2+}$ provided evidence of additive synergism.

The increased transmembrane $Ca^{2+}$ current resulting from the higher concentration gradient and the opening of the ryanodine channels of the sarcoplasmic reticulum (SR) obviously contributed to this increase in the amplitude.

The blockade of angiotensin II-induced tonic contraction after the administration of non-selective and selective blockers of T-type $Ca^{2+}$ channels unequivocally showed the role of transmembrane $Ca^{2+}$ influx in the initiation of smooth muscle contraction.

When angiotensin II binds to its membrane receptors, phospholipase C is activated resulting in the formation of inositol triphosphate, which releases $Ca^{2+}$ from SR.

It's also well known that angiotensin II causes calcium-induced calcium release in smooth-muscle cells. Angiotensin II causes depolarization and opening of VDCC, providing additional $Ca^{2+}$ influx from the extracellular fluid. Penetrating into the cell, $Ca^{2+}$ binds to ryanodine receptors and triggers supplementary $Ca^{2+}$ release from SR stores (de Gasparo *et al.,* 1992).

In the present experiment this particular calcium-induced $Ca^{2+}$ release is inhibited. The lack of tonic contraction suggested that this mechanism of intracellular calcium increase was of greater importance for the development of detrusor muscle contraction than the inositol triphosphate pathway.

In conclusion, our experimental data showed that the increase of calcium in extracellular fluid produced additive synergistic effect on angiotensin II-mediated contraction of detrusor smooth muscle strips. The calcium-induced calcium release from SR was more important for the amplitude of the tonic contraction of muscle strips than the release of calcium from the same stores induced by inositol triphosphate.

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