METHODS: The distribution pattern of Ang II and Ang II receptors in rat bladder were determined by immunohistochemistry using an ABC technique. For functional studies, bladders were harvested from anesthetized rats and placed in cold, oxygenated Kreb's solution. Bladders were cut into 2x4mm strips, mounted in perfusion chambers at 37°, placed under 2 grams of tension and equilibrated for 45 minutes. The contractile force generated in response to electric field stimulation (1-64 Hz, 10V, 5ms) and the level of spontaneous activity was measured before and after exposure to increasing concentrations of an Ang II receptor antagonist (losartan).

RESULTS: Extensive staining for AngII and its receptor were demonstrated in the rat bladder, particularly in smooth muscle bundles. The frequency and amplitude of spontaneous activity in smooth muscle tissue was completely inhibited by losartan (figure 1). Electric field stimulation resulted in a frequency dependent increase in contractile force. This response was significantly attenuated in the presence of losartan in a dose dependent manner (figure 2).

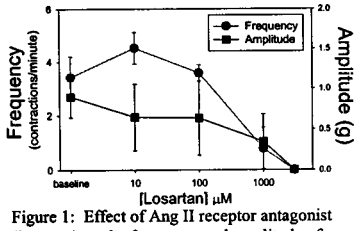

Figure 1: Effect of Ang II receptor antagonist (losartan) on the frequency and amplitude of spontaneous activity.

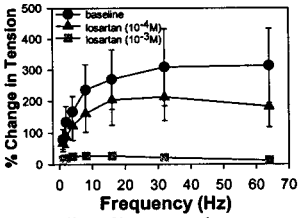

Figure 2: Effect of losartan on the response to electric field stimulation.

CONCLUSION: The extensive distribution pattern of Ang II and AngII receptor immunostaining support the concept of locally produced AngII in bladder tissue. The effect of an Ang II receptor antagonist on the bladder suggests that spontaneous activity and the response to neural stimulation are partly mediated by Ang II released from bladder smooth muscle. Thus, Ang II may play an important role in modulation of bladder smooth muscle functional response to physiologic stimuli.

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Title (type in CAPITAL LETTERS, leave one blank line before the text):

EVIDENCE OF GAP JUNCTIONS IN THE URINARY BLADDER

AIMS OF STUDY: Gap Junctions are transmembrane channels that link adjacent cells and facilitate intercellular communication. Formed by connexin proteins, these specialized structures play an important role in electrical and chemical coupling between neighboring cells in many tissues. Previous studies have shown that gap junctional activity plays a role in regulating vascular smooth muscle tone, coordinating uterine contractile activity during parturition and modulating pharmaco-mechanical coupling in cavernosal smooth muscle cells [1, 2, 3]. However, their presence in detrusor smooth muscles has not been established. The aim of this study was to determine the role of gap junctions in detrusor smooth muscle function.

METHODS: Urinary bladders from normal rats (Sprague-Dawley) were harvested and placed immediately in Kreb's solution. Longitudinal strips of bladder tissue (2mm x 4mm) were suspended

in organ baths containing Kreb's solution maintained at 37°C. The amplitude and frequency of spontaneous activity as well as the response to field stimulation were determined. The response of detrusor tissue strips to heptanol (10^{-9} to 10^{-3}M), a gap junction uncoupler, or tetraethylammonium (TEA: 10^{-6} to 10^{-3}M) a gap junction up-regulator were obtained. A carbachol dose response curve (10^{-9} to 10^{-5}M) was generated before and after exposure to heptanol (10^{-4} to 10^{-3} M).

RESULTS: Electric field stimulation produced a consistent, frequency dependent increase in contractile force. The contractile response to electric field stimulation was significantly attenuated in the presence of heptanol in a dose dependent manner (figure 1). Exposure to heptanol also resulted in a significant decrease in the frequency and amplitude of spontaneous activity (figure 2). In addition, the carbachol dose response was significantly attenuated following heptanol exposure (figure 3). Conversely, TEA increased the frequency and amplitude of spontaneous activity.

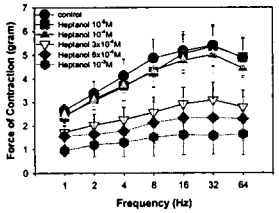

Figure 1: Effect of heptanol on the response to electric field stimulation.

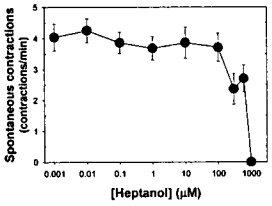

Figure 2: Effect of heptanol on frequency of spontaneous activity.

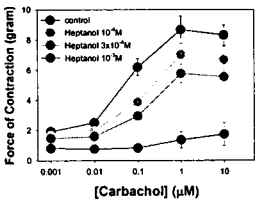

Figure 3: Effect of heptanol on carbachol dose response curve.

CONCLUSION: The effect of heptanol on spontaneous activity and on the responses to field stimulation and carbachol suggest that gap junctions may play an important role in bladder contractility by facilitating propagation of electrical signals. Furthermore, changes in gap junctional activity may contribute to altered detrusor smooth muscle function observed in detrusor instability or detrusor underactivity. Studies to identify and localize specific gap junction proteins are ongoing to support these findings.

1. J Pharmacol Exp Ther. 266:1054-1065, 1992.
2. Am J Physiol. 249:C20-C31, 1985.
3. Life Sci 49:PL195-200, 1991.

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Title (type in CAPITAL LETTERS, leave one blank line before the text):

ALTERATIONS IN THE PROFILE AND TRANSLOCATION OF PROTEIN KINASE C (PKC) IN NORMAL AND DIABETIC DETRUSOR MUSCLE OF RAT.

INTRODUCTION- Published data suggest that diabetes mellitus (DM) complications in the lower urinary tract (LUT) may be due to specific malfunctions in signal transduction pathways in LUT. PKC has been recognized as a prominent intracellular signaling pathway that modulates the effects of cholinergic and adrenergic neurotransmission in many tissues/cells. The goal of the current study was to evaluate the changes in profile and translocation of PKC in the bladder of diabetic rat.

METHODS- Detrusor muscle strips from a transgenic rat model of DM (n=15) and similar age-matched