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NITRIC OXIDE REGULATES RABBIT BLADDER BLOOD FLOW AND MICROCIRCULATION RESISTANCE

Aims of Study: Chronic arterial insufficiency has been shown to cause structural damage in bladder epithelium, fibrosis and non-compliance of the bladder wall as well as increased contractility of bladder smooth muscle. While changes in bladder blood flow are of great pathophysiologic importance, bladder microcirculatory physiology and its neuroregulatory mechanisms are poorly understood. The aim of this study was to determine the roles of the adrenergic, cholinergic and nitric oxide (NO) pathways in the regulation of bladder blood flow and microcirculation resistance (MCR) at rest and during filling.

Methods: In anesthetized male New Zealand white rabbits (3.5-4 kg, n=18), a midline abdominal incision was made and the bladder was exposed. Bladder blood flow was measured by a laser Doppler flowmeter. The laser Doppler probes were placed directly into the detrusor muscle at the bladder dome and at the base. An 18 gauge angiocatheter was inserted through the bladder wall for measurement of intravesical pressure. A 3 F catheter placed through the urethra was used to fill the bladder with normal saline. Simultaneous measurements of arterial pressure, bladder wall blood flow at the dome and base and intravesical pressure were obtained at rest and at intravesical volumes of 25 and 50 ml before and after treatment. Changes in bladder MCR were determined by the ratio of mean arterial pressure to blood flow. Bladder blood flow and MCR were continuously recorded before and after intravenous administration of the following drugs: cholinergic blocker atropine, alfa-adrenergic blocker phentolamine, beta-adrenergic blocker propranolol, NO syntheses blocker NNA and NO precursor L-arginine.

Results: IV administration of atropine tended to increase bladder blood flow at intravesical volumes of 25 and 50 ml but its effect was not statistically significant. IV phentolamine caused a significant decrease in bladder MCR in at intravesical volume of 50 ml without affecting blood flow, possibly due to simultaneous decrease in systemic arterial pressure. Propranolol caused a significant increase in bladder blood flow at the base at rest and at intravesical volume of 25 ml but not at intravesical volume of 50 ml. At the bladder dome, however, there were significant increases in MCR and and decreased blood flow after propranolol. One possible mechanism of this increase may be increased alpha-adrenergic tone in the presence of beta-adrenergic blockade. Another possibility is increased bladder wall tension at the bladder dome but not at the base. It is well established that beta-adrenoceptors are mainly present at the dome of the bladder so their blockade will lead to increased smooth muscle tone, compression of the microvasculature and increased MCR at the dome of the bladder. Since propranolol does not affect MCR at the bladder base the mechanical shift of blood flow from the dome to the base may explain the increased blood flow at the bladder base. This is supported by data showing that total bladder blood flow (bladder dome + bladder base blood flow) after propranolol was unchanged when compared with control group. This is also consistent with observations by Andersson in 1985 showing unchanged total blood flow after propranolol (they measured total venous blood output from the bladder). IV administration of NNA caused significant increase in bladder MCR and decreased bladder blood flow. At the bladder dome IV administration of L-arginine caused a significant increase in blood flow and decrease in MCR at all intravesical volumes (Fig).

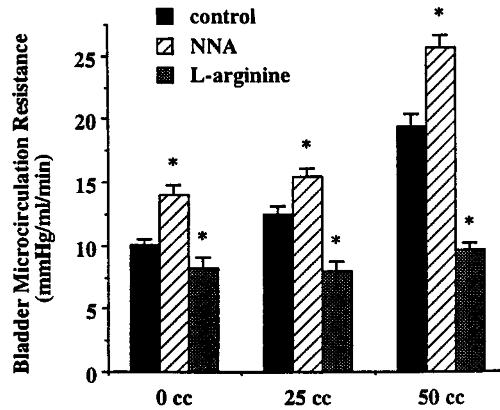

Figure. Microcirculation resistance at the bladder dome after intravenous administration of NNA and L-arginine.

<u>Conclusions:</u> Bladder microcirculatory resistance and hence bladder blood flow are primarily regulated by the NO pathway.

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SENSORY MECHANISMS IN THE URINARY BLADDER: ADAPTATION TO CHANGES IN URINARY SODIUM CONCENTRATION

Aims of the Study: A proposal, which describes the mechanisms by which the urinary bladder detects its degree of distension, has been recently published (1). In brief urothelial cells carry mechanosensitive sodium channels in their apical membranes. These channels change the transport of sodium as they are stretched, and this results in release of adenosine 5'-triphosphate (ATP) from the basolateral surfaces of the cells on to the underlying sensory nerves. The aim of the present study is to discover how such a sodium-driven sensory mechanism can adapt to changes in urinary sodium concentration.

Methods: Isolated urinary bladders from Dutch rabbits of either gender were mounted in Ussing chambers. The short-circuit current (SCC), which measures the transepithelial sodium flux, and the transepithelial potential (TEP) differences, were measured and recorded on a flat bed recorder. The bladder was bathed in normal Krebs' solution at 37°C and oxygenated with 95% oxygen and 5% carbon dioxide. In experiments in which the sodium concentration was changed the osmolarity of the medium was maintained by substitution of sodium by N-methyl D- glucamine. The composition of the medium on both sides of the membrane was kept identical so that potential differences between the electrodes were not introduced. Amiloride at 1x 10^{-5} M was used to measure the magnitude of amiloride sensitive sodium flux across the bladder wall.
For examination of the tissue by electron microscopy, the bladders were mounted in Ussing chambers as above with horse-radish peroxidase (HRP) present on the urinary surface. Sodium concentrations were varied and the tissue rapidly fixed, reacted with diaminobenzidine, embedded in Spurr's resin, and sectioned.

Results: Normal rabbit bladders change their TEP by about 30% in response to a 3.5cm of water pressure change (1). This change is due to alterations in sodium transport from the urine into urothelial cells. If the signal for ATP release from the basolateral surface of the cells is the intracellular sodium concentration, clearly at high concentrations of urinary sodium, more sodium would enter for a given pressure stimulus. The normal urinary sodium concentration in rabbits on a laboratory diet is 69 ± 30mM (2). Can the cells regulate their sodium entry as the external sodium concentration changes?
Initial experiments showed that if all the sodium on both surfaces of the membrane was substituted by N-methyl-D-glucamine, both SCC and TEP fell to a low level. Sometimes the TEP changed polarity indicating that potassium was leaving the cells into the urine. Short circuit current and TEP measurements made in regular sodium containing Krebs' were greater following previous preincubation in sodium free Krebs'. This suggests that low sodium concentration results in additional sodium channels being added to the membrane. This phenomenon had previously been demonstrated in another context by other authors (3).
If bladders were incubated at zero sodium for one hour and 25mM sodium added in a stepwise fashion the SCC and TEP increased in a correspondingly stepwise fashion, until about 60mM when they both rose but fell back rapidly to the pre-existing level. When SCC was plotted against external sodium concentration a hyperbolic relationship was seen. One explanation of this was that sodium channels were removed from the apical membrane as the external sodium concentration increased. An identical experiment was performed on bladders pre-incubated with brefeldin at 1x 10^{-6} M. Under these circumstances the increase in SCC and TEP normally seen in zero sodium was reduced by approximately 50%; subsequent stepwise increase in sodium concentration still showed a hyperbolic relationship but at a lower level. This indicated that removal of sodium channels was unaffected by brefeldin, but that vesicular insertion was inhibited by the drug.