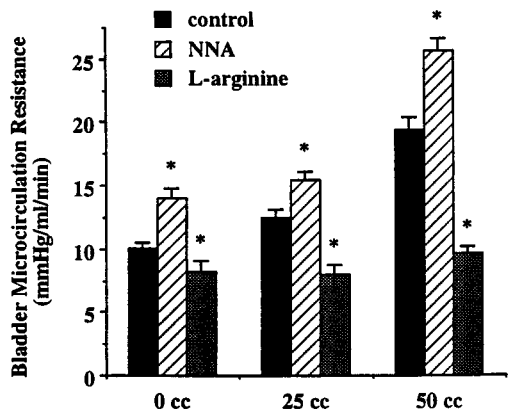

Figure. Microcirculation resistance at the bladder dome after intravenous administration of NNA and L-arginine.

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

77

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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.

If the bladders were incubated at zero sodium for one hour so that maximum numbers of sodium channels were inserted into the apical membrane, and the sodium concentration suddenly jumped to 100mM, the SCC and TEP fell in an exponential fashion. The half time of this process was 26 minutes, indicating that the channels were being removed under these conditions.
Rabbit bladders were incubated in zero sodium for one hour and HRP placed on the urinary surface. Then the sodium concentration was increased to 100mM and the tissue was incubated for one hour under these conditions. The tissue was subsequently fixed and processed for electron microscopy. The images revealed large intracellular vesicles that contained HRP.

Conclusions: The hydrostatic pressure sensing mechanism in urinary bladder is driven by sodium ions entering urothelial cells through mechanosensitive channels. The numbers of such channels is inversely related to the urinary sodium concentration. The movement of vesicles in and out of the plasma membrane effects these changes. This vesicular movement is asymmetrical as membrane insertion is inhibited by brefeldin, which does not affect removal of vesicles from the apical membranes.

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78

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THE DISTRIBUTION OF $P2X_1$ AND $P2X_3$ RECEPTORS IN THE RAT AND HUMAN URINARY BLADDER.

Aims of the Study: Adenosine and adenosine 5'-triphosphate (ATP) have well recognised functions as neuromodulator and neurotransmitter, respectively, in smooth muscle preparations (1,2,3,4,6). Recent evidence has shown that ATP may have both a sensory role in processing physiological information in the urinary bladder (5) and cause bladder contractions (2, 9) and that these effects are likely to be mediated by ATP receptors, namely $P2X_1$ and $P2X_3$. This study, therefore, set out to address this question using subtype-specific antibodies to localise these receptors in the human and rat urinary bladder.

Methods: All rat experiments were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC). All human experiments were carried out in accordance with the declaration of Helsinki. Each patient understood the procedures performed and gave their explicit consent. Standard immunocytochemical methods have been used to identify the distribution of $P2X_1$ and $P2X_3$ receptors.

Results:
Light microscopy
In both the rat and human urinary bladder the suburothelium and urothelium were evident and were folded in both species. There was a clear demarcation between the detrusor muscle and the urothelium in the rat urinary bladder. The suburothelium, in both species, was composed of connective tissue, blood vessels and nerves. It is in this region that sensory nerves are expected to project to the basal layers of the urothelium. However, it was not possible to see bundles of nerves in this region in the sections stained with haematoxylin and Eosin.
The urothelium of the human bladder is composed of 4-5 cell layers, whereas that of the rat consists of only 2-3 cell layers. A row of blood vessels was located in the suburothelium in both species but it was most prominent in the rat. The underlying detrusor muscle in both species was composed of a thick layer of muscle bundles embedded in a band of connective tissue with a relatively sparse vasculature.

Immunofluorescence confocal microscopy using the $P2X_1$ and $P2X_3$ receptor antibodies
The labelling pattern of the smooth muscle cells of the detrusor muscle of the rat and human bladder, for the $P2X_1$ receptor was identical to that observed on smooth muscle cells of the rat vas deferens (positive control for $P2X_1$). Intense labelling was present over the sarcolemma of the smooth muscle cells. No labelling was observed over the myoepithelial cells or any other structure in the sub-urothelium or the urothelium.
There was no detectable labelling of the detrusor muscle in either species using the $P2X_3$ receptor antibody and only low levels of immunofluorescence were seen over the myothelium. However, strong labelling was present in the basal region of the urothelium of both species. The strong labelling by the $P2X_3$ receptor antibody was present on both the nerve fibres and over the urothelium itself. When the images are compared, the $P2X_3$ receptor labelling, appears to lie in between the cells more prominently in the human urothelium than in that of the rat. In the overlay micrograph of the rat urothelium, the localisation of the $P2X_3$ receptor antibody can clearly be separated from the cell nuclei, stained with propidium iodide, and is therefore restricted to the cytoplasm and cell surfaces.
Conclusions: Previous studies suggested that ATP has a sensory role both in rabbit (5) and rat bladders. $P2X_1$ receptors are present in the urinary bladder of both the rat and the rabbit. The significance of $P2X_1$ receptors in the human bladder is unclear. $P2X_3$ receptors were also localised on fibres in the suburothelial layer where labelling for calcitonin gene-related peptide (CGRP) was also seen. This distribution pattern suggests that these receptors are involved in sensory transmission in the rat and human urinary bladder. However, labelling for $P2X_3$ receptors was also found in unexpected places, such as within the urothelium of the human and the rat urinary bladders.
Unlike other species, the normal human bladder does not appear to have a non-cholinergic excitatory component to neuronal stimulation (10). However, atropine-resistant contractions have been observed in functionally disturbed, hypertrophied human bladders, where ATP mimics the responses to nerve stimulation (7, 11). It appears that the unstable hypertrophied human bladder, which is present in patients with some types of incontinence, exhibits increased sensitivity to ATP and possibly acquires an ATP component to neuronal stimulation which is absent from the normal bladder. Furthermore, other authors (8) have proposed a nociceptive role for ATP in sensory transmission. At present the roles of the receptors we have demonstrated in rat and human bladders, as physiological or nociceptive mediators cannot be distinguished. However, it is clear that $P2X_1$ and $P2X_3$ receptors are both present and are likely to have different functions. This suggests that further work needs to be undertaken to clarify the role of ATP in the pathologically disturbed urinary bladder and the normal bladder.