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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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.

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EVALUATION OF THE SITE OF ACTION OF PROSTANOIDS IN THE MICTURITION REFLEX PATHWAY OF THE RAT.

Aims of Study:

Prostanoids increase the frequency of spontaneous activity of the detrusor smooth muscle, contribute to basal tone and lower the threshold volume for micturition [1,2]. The aim of the present study was to identify the site(s) of action of prostanoids in the micturition reflex by examining the effects of the cyclo-oxygenase inhibitor, indomethacin, on responsiveness of rat urinary bladder in vitro and in vivo.

Methods:

In vitro studies: Urinary bladders were removed from rats that had been euthanized using CO_2 asphyxiation. Bladders were opened along their ventral surface and longitudinal strips of muscle (2-3 mm in diameter) were prepared. Strips were mounted in 5 ml tissue baths containing oxygenated (95% O_2: 5% CO_2) modified Krebs buffer solution (mM:NaCl, 118.2; Dextrose, 10.0; KCl, 4.6; KH_2PO_4, 1.2; $MgSO_4 7H_2O$, 1.2; $NaHCO_3$, 24.8; $CaCl_2$, 2.5; 37°C, pH of 7.4.) and allowed to equilibrate at a resting tension of ~2 g for 60 min. Indomethacin (1 μM) or vehicle (0.5 % Na_2CO_3) were applied to tissues and equilibrated for 60 min. The effects of treatment were evaluated on both basal tone and spontaneous myogenic activity of the preparations to identify potential post-junctional effects of prostanoids.

In vivo studies: Two in vivo models were utilized. The electrically-stimulated pithed rat model (ESPR) provides a means of evaluating the effects of drugs on the efferent limb of the micturition reflex [3]. The volume-induced bladder contraction model (VIBC) [4] involves both the afferent and efferent limbs of the micturition reflex and, in conjunction with the ESPR model, provides a means of evaluating the effects of drugs on the afferent limb of the micturition reflex. For the ESPR model, rats were anesthetized with pentobarbital sodium (60mg/kg, i.p) and the femoral artery and both femoral veins were cannulated for the measurement of blood pressure and administration of drug and fluid, respectively. The bladder was cannulated via the external urethral orifice and connected to a pressure transducer and infusion pump. An insulated steel pithing rod was inserted through the ocular orbit and into the spinal column, dissociating the central nervous system from the peripheral nervous system. The tip of the rod, which was not insulated, was placed 14 cm down the length of the spinal cord such that electrical stimulation of the rod activated nerves in the L6-S2 spinal regions, eliciting contraction of the detrusor smooth muscle via activation of pre-ganglionic efferent fibers. For the VIBC model, rats were anesthetized with urethane (1.5 g/kg, s.c.) and the carotid artery and femoral vein were cannulated for the recording of blood pressure and administration of drug, respectively. The bladder was cannulated via the external urethral orifice and connected to a pressure transducer and infusion pump. Rhythmic bladder contractions were induced in these animals by the infusion of 1.5 ml of warm saline into the bladder. In both