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ATP is released from guinea pig ureter epithelium on distension

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Knight, G. E., P. Bodin, W. C. De Groat, and G. Burnstock. ATP is released from guinea pig ureter epithelium on distension. *Am J Physiol Renal Physiol* 282: F281–F288, 2002; 10.1152/ajprenal.00293.2001.—Distension of the perfused guinea pig ureter at pressures from 20 to 700 cmH_2O increased the amount of ATP released from the epithelium in a pressure-dependent manner. During basal perfusion (40 μl/min), the perfusate contained 10 pmol/ml ATP; this increased 10- to 50-fold at various distending pressures. ATP was released from epithelial cells during distension as mechanical removal of the urothelium blocked release. No lactate dehydrogenase was detected in the perfusate, and scanning electron microscopy confirmed an intact urothelium after distension. ATP was not released due to the activation of stretch-activated channels, as gadolinium (10 μM) failed to affect ATP release. Glibenclamide (10 μM), known to inhibit two members of the ATP-binding cassette (ABC) protein family, did not affect ATP release after distension; nor did verapamil (10 μM). In contrast, both monensin (100 μM) and brefeldin A (10 μM), which interfere with vesicular formation or trafficking, inhibited distension-evoked ATP release, which was Ca^{2+}-dependent. This suggests that ATP release from the ureter epithelium might be mediated by vesicular exocytosis. The role of ATP released by distension of hollow visceral organs is discussed in relation to the concept of purinergic mechanosensory transductions, with special reference to nociception and the activation of $P2X_3$ receptors on the subepithelial sensory nerves.

adenosine 5′-triphosphate; luciferin-luciferase; nociception; urothelium; vesicular exocytosis

ATP IS RAPIDLY RELEASED from vascular endothelial cells during periods of increased flow, shear stress, and hypoxia (5, 6, 33). After release, ATP can activate endothelial P2Y receptors to stimulate the synthesis of nitric oxide (NO) (40), which in turn produces vasodilatation of perfused systems such as the rat pulmonary vascular bed (25) and rabbit and guinea pig coronary beds (49, 51).

ATP is also released from both human and rabbit red blood cells (RBCs) in response to mechanical deformation (48). The ATP is then able to exert a regulatory effect on vascular resistance via stimulation of NO. ATP is a highly charged molecule and does not readily cross cell membranes. It is thought that ATP leaves RBCs via one or more ion channels in the ATP-binding cassette (ABC) family (18). Although the activity of this superfamily as ATP transporters is questioned, some reports claim that ATP is transported directly via these proteins (1, 45), whereas others disclaim this hypothesis (24, 31).

There is also increasing evidence for the release of ATP from epithelial cells. Ferguson and coworkers (20) found that small changes in hydrostatic pressure resulted in the release of ATP from the urothelium of the rabbit urinary bladder. They proposed that ATP released from the urothelium could act as a sensory mediator of the degree of distension. Similarly, hypotonic stimulation of cultured ocular ciliary epithelial cells increased the extracellular ATP concentration by a factor of three (35). This may be important in the pathogenesis of glaucoma, because adenosine and ATP have been implicated in the regulation of ciliary epithelial Cl^- conductance and formation of aqueous humor (12, 17, 35).

ATP has been found to be released from human tracheal epithelial cells via volume-sensitive Cl^- channels in response to hypotonic shock (38). It is then degraded rapidly to adenosine, which in turn modulates the anion channels. Such autocrine signaling via the release of ATP has also been found to occur in rat hepatocytes (55).

Recently, Burnstock (9) suggested that ATP is released from epithelial cells lining structures that could be considered either "sacs" (e.g., bladder and lung) or "tubes" (e.g., ureter, gut, and salivary and bile ducts). He suggested that deformation of the epithelial cells resulting from distension leads via a selective transport system to the release of ATP, which then acts on subepithelial sensory nerves. ATP has been shown to activate sensory neurons (8, 10, 11) via a novel ligand-gated ion channel designated as the $P2X_3$ receptor. The $P2X_3$ homomultimer and $P2X_{2/3}$ heteromultimer receptor subtypes have been identified on nociceptive sensory neurons (11, 14, 30), specifically, on subpopula-

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tions of cell bodies within the dorsal root, trigeminal, and nodose ganglia (7, 32, 53, 54). Thus a link between ATP and the sensation of pain has been defined.

The aim of this study was to determine whether distension of the ureter (a tube) results in the release of ATP from the epithelial layer. The effect of blockers of ATP transport mechanisms and stretch-activated channels were also studied to investigate the mechanism by which ATP is released in response to mechanical stimulation.

METHODS

General procedures. Male guinea pigs (body wt 275–325 g) were killed by CO_2 asphyxiation, and death was confirmed by cervical dislocation according to U.K. Home Office regulations covering Schedule One procedures. Both ureters were isolated from the kidney to the bladder and connective tissue and fat were carefully removed. The proximal end of each ureter was marked with a loose silk tie and placed into physiological saline solution of the following composition (in mM): 133 NaCl, 4.7 KCl, 16.4 $NaHCO_3$, 0.6 $MgSO_4$, 1.4 NaH_2PO_4, 7.7 glucose, and 2.5 $CaCl_2$; pH 7.4.

The ureters were transferred to a petri dish embedded with dental wax into which noncorrodible metal staples had been previously positioned to keep the ureters straight and prevent excessive movement. A catheter was inserted into the proximal end of each ureter and secured with silk thread, and a tube of similar dimensions was attached to the distal end. The catheters were used to perfuse the ureter with physiological saline. The solution and the petri dish were continuously gassed with 95% O_2-5% CO_2. All experiments were carried out at room temperature.

Distension experiments. The ureter was perfused using a peristaltic pump (Watson-Marlow, Fainmouth, U.K.) via a pressure transducer (model P23 ID, Gould, Oxnard, CA) at a constant flow rate of 40 μl/min. Pressure was monitored on a Grass ink-writing oscillograph. The ureter was initially perfused for 45 min before the experiment began.

The perfusate was collected in microcentrifuge tubes for 5 min over a 20-min period and stored on ice to measure basal ATP levels. After 20 min, the outflow tube was clamped and the ureter was allowed to fill until a specific pressure (measured in cmH_2O) was reached. The pump was then stopped to prevent further distension. The contents of the ureter and outflow tube were allowed to run into a tube. The pump was started again and an additional 90 s of perfusate was added to allow collection of any perfusate still within the ureter. Two control samples (5 min at 40 μl/min) were collected before an additional distension was attempted. The ureter was distended to various pressures (20–700 cmH_2O).

Several substances [10 μM atractyloside; 10 μM brefeldin A; 10 μM 4,4′-diisothiocyanatostilbene-2,2′-disulphonate (DIDS); 30 μM dipyridamole; 10 μM glibenclamide; 10 μM gadolinium; 1 mM *N*-ethylmaleimide (NEM); 100 μM monensin; and 10 μM verapamil] were examined for possible effects on ATP release. Two distensions (150–300 cmH_2O) together with controls were performed before the addition of one of these drugs to the perfusion medium. The ureter was perfused for 30 min in the presence of the potential inhibitor before the controls and distensions were repeated. Experiments were also performed in Ca^{2+}-free, high-Mg^{2+} Krebs solution (10 mM) with EGTA (100 μM) added. Three distensions of increasing pressures (50, 100, and 200 cmH_2O) were performed with this perfusion medium after either equilibration in the Ca^{2+}-free medium for 1 or 2 h.

The effect of removing the urothelium upon distension-evoked ATP release was also examined. In some experiments, the urothelium was removed from one ureter at the beginning of the experiment by running a catheter tube along the length of the ureter and subsequently blowing air through the tube. The ureter was then subjected to similar distensions as the urothelium-intact ureter. In some experiments, a marker of cellular injury, lactate dehydrogenase (LDH; Sigma procedure 500), was measured in samples after a distension to determine whether there was damage to the urothelium during the distension. To evaluate the effect of injury, at the end of some experiments the ureters were deliberately damaged by crushing them with a pair of forceps, and the perfusate was collected.

Luciferin-luciferase assay for ATP. The quantity of ATP in the perfusate collected from the ureters was quantitated by luminometry using the luciferin-luciferase assay. Briefly, ATP standards and perfusate samples (50 μl) were pipetted into a white (nonphosphorescent) 96-well microplate. The plate was placed in a luminometer (Lucy 1, Anthos Labtec, Salzberg, Austria) and processed automatically by injection of 100 μl of luciferin-luciferase reagent (ATP monitoring reagent, Bio-Orbit, Turku, Finland) into each well and measured for 10 s. The ATP concentrations were calculated from a calibration curve constructed from the ATP standards. The detection limit was ~5 fmol of ATP per sample. None of the inhibitors used in this study or the lack of Ca^{2+} in the medium of some samples had any effect on the sensitivity of the ATP assay.

LDH assay. Using the Sigma LDH colorimetric assay procedure, in some experiments estimations of LDH activity were made from samples of perfusate taken after distension or damage to the ureter. A 50-μl aliquot of the sample was incubated at 37°C with 0.75 mM pyruvate and 1.28 mM NADH for 30 min. The reaction was stopped with 0.1 ml of Sigma color reagent and left for 20 min before addition of 5 ml of 0.4 mM sodium hydroxide. The absorbance was read at 450 nm.

Scanning electron microscopy. Ureters were also examined with a scanning electron microscope to ascertain the condition of the urothelium. Examples of ureters that had not been subjected to any distension ($n = 2$), those that had been distended ($n = 5$), and those where the epithelial layer had been removed ($n = 5$) were examined.

Briefly, specimens were fixed overnight in 2% gluteraldehyde in 0.1 M phosphate buffer at 4°C. After fixation, the ureters were cut into two longitudinal sections to expose the urothelium, were rinsed in 0.1 M phosphate buffer, and were postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer. The specimens were dehydrated through a graded ethanol series before being critical-point dried, sputter-coated with a 30-nm-thick film of gold, and viewed on a Jeol 5410LV scanning electron microscope.

Drugs and chemicals used. Atractyloside, brefeldin A, DIDS, dipyridamole, EGTA, monensin, NEM, gadolinium, glibenclamide, gluteraldehyde, luciferin-luciferase, LDH, NADH, osmium tetroxide, and verapamil were all obtained from Sigma (Poole, UK). All chemicals were dissolved in distilled water except brefeldin A and monensin, which were dissolved in methanol to form a stock solution (0.1 M) and were subsequently dissolved in Krebs solution.

Statistical analysis. A control sample of perfusate (5-min collection at 40 μl/min) immediately preceding distension and perfusate samples taken during and immediately after distension were measured for ATP (pmol/ml).

Intraureteral distension pressures were grouped into 50-cmH_2O increases in pressure, up to 200 cmH_2O (i.e., 0–50, 50–100, 100–150, and 150–200 cmH_2O), followed by pres-

sures within the 200–300-cmH_2O range, and finally pressures >300 cmH_2O up to a maximum of 700 cmH_2O. Within these groups and in controls, the concentration of ATP (pmol/ml) is expressed as the mean release ± SE (n), where n represents the number of animals. These were then analyzed by paired Student's t-test using GraphPad Prism software to identify concentrations of ATP that were significantly different from the control; $P < 0.05$ was taken as significant.

In the pharmacological experiments, the concentration of ATP released in two distensions before the addition of the drug were compared with two distensions (150–300 cmH_2O) in the presence of the drug. These were analyzed using a paired Student's t-test, with $P < 0.05$ being taken as significant.

RESULTS

Distension experiments. Distending the ureter by as little as 20 cmH_2O caused a significant increase in the concentration of ATP in the perfusate compared with the basal level of ATP at a flow rate of 40 μl/min. The basal concentration of ATP that was recorded in the control samples was 9.9 ± 1.1 pmol/ml (n = 83 observations). As the distension pressure increased, the concentration of ATP was also significantly increased (range 100–600 pmol/ml) compared with the control value immediately before the distension ($P < 0.05$; Fig. 1*A*). At the maximum distension pressure used in these experiments (>650 cmH_2O), the ATP concentration was 620.3 pmol/ml. Multiple distensions of approximately equal pressures (50–100 cmH_2O) produced similar distension-evoked increases in ATP concentration (Fig. 1*B*).

Various agents were tested as potential inhibitors of distension-evoked ATP release. Those found to be without effect included glibenclamide (10 μM, n = 7), which was perfused for 30 min at a concentration known to suppress ATP release in blood vessels (22). Glibenclamide failed to affect ATP release during two consecutive distensions (150–300 cmH_2O) compared with two similar distensions before the addition of glibenclamide (Fig. 2*A*). Similarly, gadolinium (10 μM, n = 5; Fig. 2*B*), NEM (1 μM, n = 4; data not shown), DIDS (10 μM, n = 3; data not shown), dipyridamole (30 μM, n = 3; data not shown), atractyloside (10 μM, n = 3; data not shown), and verapamil (10 μM, n = 3; data not shown) also failed to affect the amount of ATP released during distension.

Both monensin (100 μM, n = 7) and brefeldin A (10 μM, n = 6) significantly ($P < 0.05$) inhibited the amount of ATP released from the ureters upon distension (150–300 cmH_2O) after incubation of the inhibitor for 30 min compared with the amount of ATP released upon distension before incubation of the inhibitor (Fig. 2, *C* and *D*, respectively).

Removing Ca^{2+} from the perfusion medium (in the presence of 100 μM EGTA) that had equilibrated for 1 h before distension failed to affect the concentration

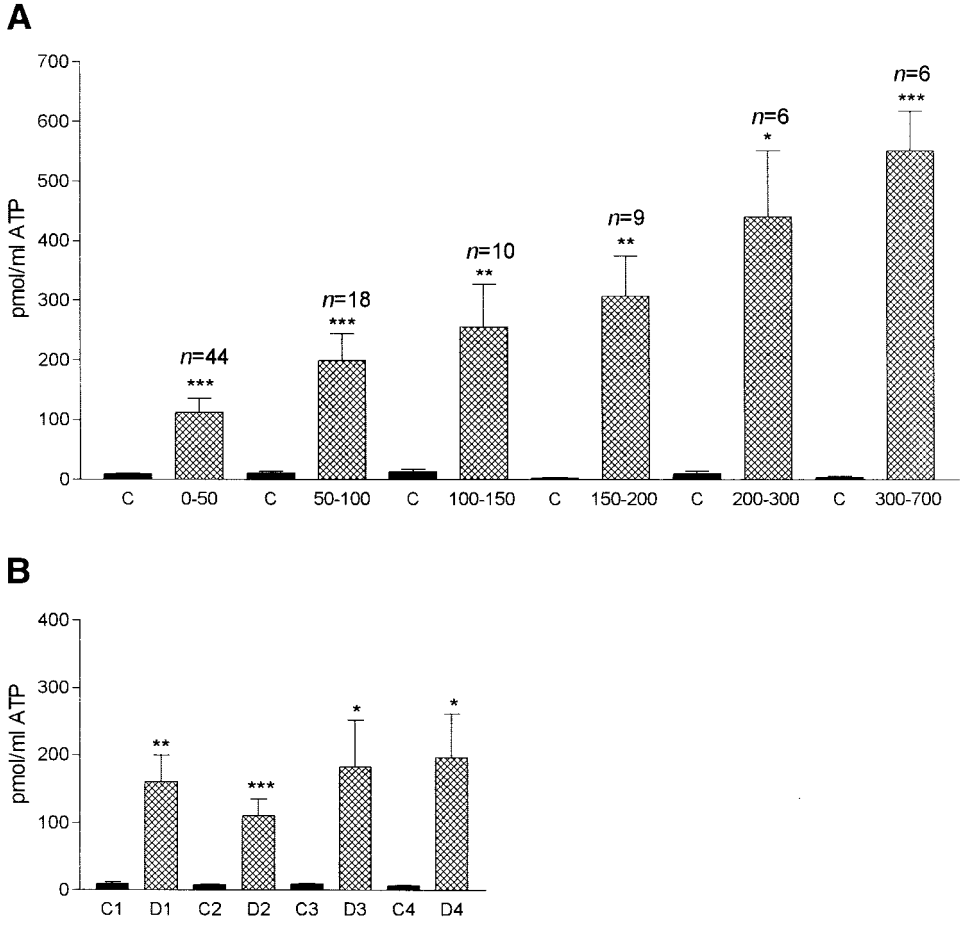

Fig. 1. Increase in the concentration of ATP in the perfusate of guinea pig ureters. *A*: release of ATP at increasing intraureteral pressures (up to 700 cmH_2O) preceded by a control sample (C, control; perfused at 40 μl/min). *B*: release of ATP during four consecutive distensions (D1-D4, all n = 17) of approximately equal distension pressures (50–100 cmH_2O), each distension preceded by a control sample (C1-C4, controls; perfused at 40 μl/min). Each bar represents the mean ATP release (pmol/ml) ± SE (n). Statistical significance was assessed using paired Student's t-test; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

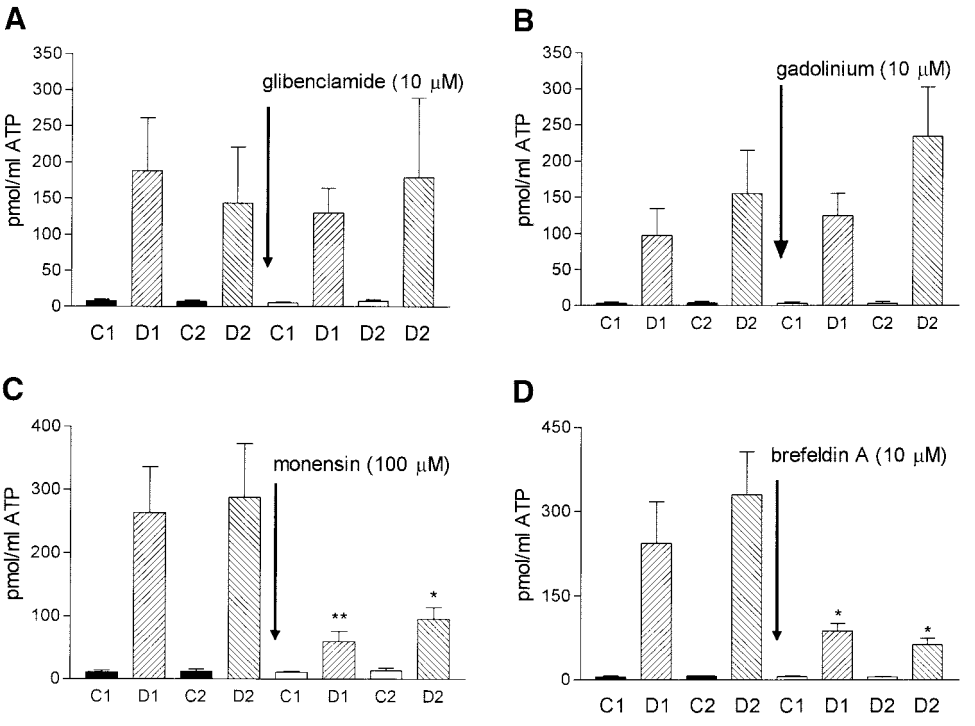

Fig. 2. Effect of various substances on the distension-evoked release of ATP in guinea pig ureters. Two distensions (D1 and D2, 150–300 cmH_2O) were preceded by two control samples (C1 and C2, perfused at 40 μl/min) before and after treatment with potential blocking agents. *A*: release of ATP during D1 and D2 before and after incubation of the perfusion medium with glibenclamide (30-min incubation, $n = 7$). *B*: release of ATP during D1 and D2 before and after incubation of the perfusion medium with gadolinium (30-min incubation, $n = 5$). *C*: release of ATP during D1 and D2 before and after incubation of the perfusion medium with monensin (30-min incubation, $n = 7$). *D*: release of ATP during D1 and D2 before and after incubation of the perfusion medium with brefeldin A (30-min incubation, $n = 6$). All bars represent the mean ATP release (pmol/ml) ± SE (n). Statistical significance between the ATP release before and after incubation with the blocking agent was assessed using paired Student's t-test; $^*P < 0.05$; $^{**}P < 0.01$.

of ATP released during distension by increasing pressures up to ~200 cmH_2O. However, increasing the equilibration period in Ca^{2+}-free medium to 2 h caused a significant ($P < 0.05$) reduction in the amount of ATP released at each of the three increasing distension pressures (50, 100, and 200 cmH_2O) compared with an equivalent distension pressure in the presence of Ca^{2+} and after 1 h of equilibration in Ca^{2+}-free medium (Fig. 3).

The effect of removal of the epithelium on ATP release during distension was also investigated. Distension of those ureters devoid of urothelium ($n = 5$) did not evoke release of ATP. The concentration of ATP released after distension did not differ statistically from that before distension (Fig. 4).

Damage. The effect of mechanical injury on ATP release was evaluated by crushing ureters with forceps. The mean ATP concentration in the perfusate before and after crushing was 18.2 ± 5.3 pmol/ml ($n = 12$) and 49,582.3 ± 11,247.4 pmol/ml ($n = 12$), respectively; statistically significantly greater after crushing.

LDH assay. In some experiments ($n = 6$), in addition to assaying ATP, LDH was also estimated after distension of the ureter. After distension (650 cmH_2O), samples of perfusate consistently had LDH activity rates below or close to the 40 U/ml sensitivity levels of the assay kit with no cell lysis occurring during distension. However, after damage to the ureter by crushing with forceps, LDH was detected in the perfusate with activity in excess of 400 U/ml, which indicates that cell lysis had occurred after crushing of the ureter.

Scanning electron microscopy. Ureters that were not subjected to any procedure (control, $n = 2$) in addition to those that were subjected to distension ($n = 5$) or epithelium removal ($n = 5$) were examined by scanning electron microscopy to observe the appearance of the epithelial layer. Those ureters that had not been subjected to any experimentation had a complete epithe-

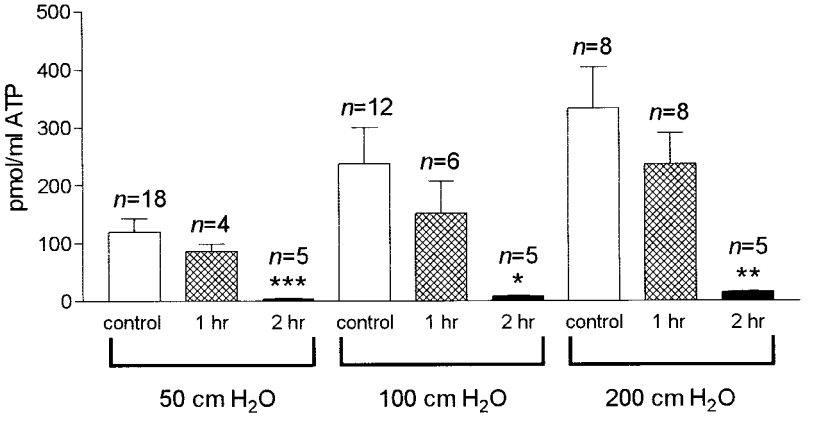

Fig. 3. Release of ATP from guinea pig ureters at increasing pressures of distension (control 50, 100, and 200 cmH_2O) in the presence of Ca^{2+} and then after incubation in Ca^{2+}-free perfusion medium (with 100 μM EGTA) for 1 or 2 h. Each bar represents the mean ATP release (pmol/ml) ± SE (n); $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

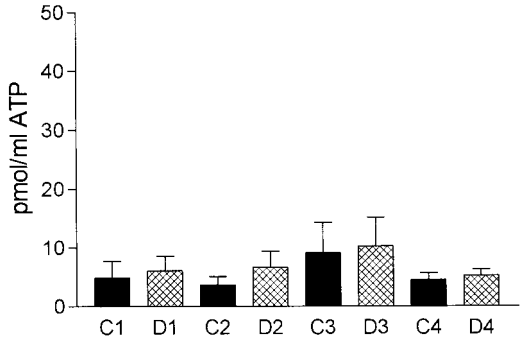

Fig. 4. Release of ATP from guinea pig ureters during four consecutive distensions (D1-D4, all $n = 5$) preceded by controls (C1-C4, perfused at 40 μl/min) after mechanical removal of the urothelium. Each bar represents the mean ATP release (pmol/ml) ± SE (n). No statistically significant differences between controls and distensions were found (paired Student's t-test $P > 0.05$).

lial layer (Fig. 5*a*). Those ureters that had been distended showed no damage to the epithelial layer (Fig. 5*b*), whereas in those where the epithelial layer had been mechanically removed, scanning electron microscopy confirmed that ~90% of the epithelium had been removed (Fig. 5, *c* and *d*).

DISCUSSION

The aim of this study was to determine whether distending the guinea pig ureter resulted in the release of ATP. The results have conclusively shown that increasing the intraluminal pressure of the ureter by as little as 20 cmH_2O significantly increases the concentration of ATP in the perfusate above the basal levels. Distension to 50 cmH_2O resulted in the release of over 100 pmol/ml ATP, a 10-fold increase compared with the basal release of ATP. The concentration of ATP released upon distension was found to be pressure-dependent, and within the time frame of the experiments the concentration of ATP released did not decline with repeated distensions.

It seems likely that ATP released during distension originated in the epithelial cells, because mechanical removal of the urothelium abolished pressure-dependent ATP release. The method that was used to remove the epithelium left the ureter smooth muscle intact (as observed by scanning electron microscopy). Scanning electron microscopy also confirmed that after distension the epithelium was undamaged. Similarly, the assay for LDH showed that cell lysis did not account for the ATP released after distension.

ATP release from cells, excluding cell rupture, can occur as a result of activation of stretch-activated channels, the opening of channel-like pathways mediated by ABC proteins (19, 26), or as a result of vesicular exocytosis. The possibility that one of these mechanisms is responsible for distension-evoked ATP release from the ureter was also investigated.

It is unlikely that ATP is being released after activation of stretch-activated channels, because gadolinium, which has been found to inhibit such channels in bacteria, *Xenopus* oocytes, and some mammalian cells (3, 41, 56), had no effect on ATP release after distension.

The ABC proteins are a diverse superfamily of structurally related membrane proteins with a common intracellular site that bind and hydrolyze ATP (18). These proteins mediate the selective movement of solutes across biological membranes (26) by utilizing ATP as an energy source for the translocation. It is currently thought that ATP transport across the plasma membrane is associated with the activity of these proteins (42). Several of these proteins have been identified as ion channels or having the ability to regulate intrinsic channel activity. Of the many ABC proteins identified, the cystic fibrosis transmembrane regulator

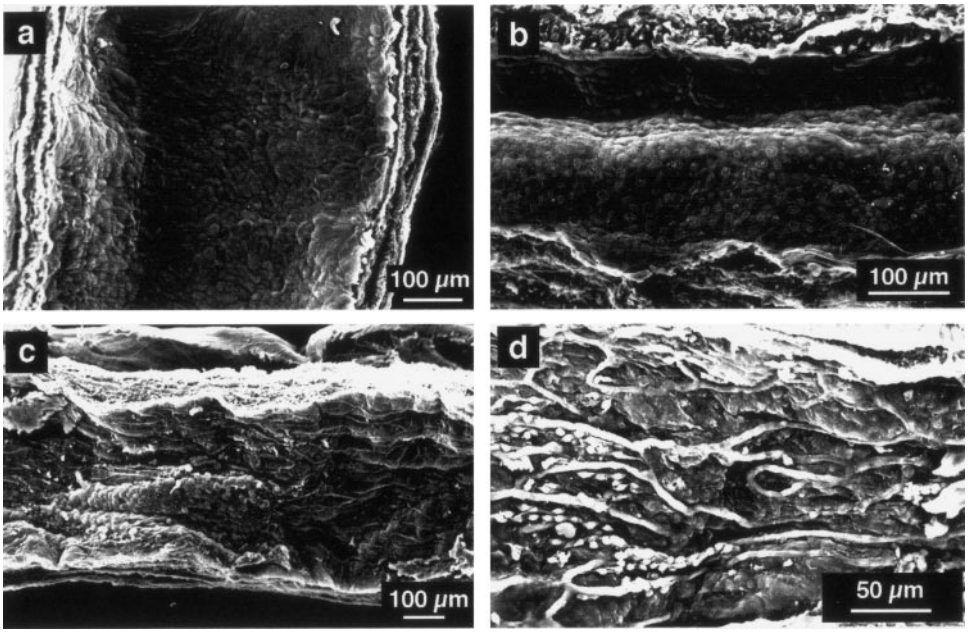

Fig. 5. Scanning electron microscopy of longitudinal sections of ureters displaying the urothelium. *a*: a control ureter that had not undergone any distension shows normal urothelium coverage. *b*: a ureter that had undergone several distensions. The urothelium is intact. *c*: a ureter from which the urothelium had been removed before the experiment and then had undergone several distensions. Note that ~90% of the urothelium has been removed. *d*: higher magnification of a ureter from which ~90% of the urothelium had been removed before the experiment.

(CFTR), the sulfonylurea receptor, and the multidrug resistance protein (MDR) have been identified on vascular endothelial cells (16, 28, 50).

Glibenclamide has many biological effects. It is able to inhibit two members of the ABC protein family, namely, the sulfonylurea receptor (which is an ATP-sensitive potassium channel) and the CFTR protein (22, 46). In this study, glibenclamide had no inhibitory effect on ATP release in response to distension, and it is therefore unlikely that these proteins are involved in the release mechanism.

Verapamil has been shown to inhibit the volume-activated Cl^- channel that is thought to be linked to P-glycoprotein expression (18). Similarly, DIDS failed to inhibit distension-evoked ATP release, which is also thought to act as a Cl^- channel blocker in addition to its several other properties (35). This is the third member of the ABC protein family. Because verapamil and DIDS failed to inhibit distension-evoked ATP release from the ureter, it would appear that ABC proteins are unlikely to facilitate ATP-release in this tissue.

The involvement of nucleoside and nucleotide transporters has also been ruled out because dipyridamole [shown to inhibit nucleoside transport in human erythrocytes (2)] and atractyloside [an inhibitor of mitochondrial ADP/ATP carrier (21)] had no effect on the amount of ATP released upon distension.

The inhibitory activity of both monensin and brefeldin A upon distension-evoked ATP release implies that ATP is being released by vesicular exocytosis. Monensin is an inhibitor of vesicular formation from the Golgi apparatus (13) and brefeldin A disrupts vesicular trafficking by inhibiting protein transport from the endoplasmic reticulum to the Golgi apparatus (23, 34). Both of these agents significantly inhibited distension-evoked ATP release from the ureter, which implies that ATP is being released by vesicular exocytosis. Although this evidence supports the hypothesis of vesicular release, it must also be noted that during the course of the investigation an inhibitor of vesicular fusion with the plasma membrane, namely, NEM (4, 44), was found to be inactive against distension-evoked ATP release. There was a trend toward an increase in the amount of ATP released upon distension in the presence of this agent. However, this agent is also known to have other activities in addition to its effect on vesicular fusion as a monovalent ion-selective ionophore (36), and may in fact be toxic to the tissue. This could account for its lack of effect and indeed the appearance of an increase in ATP release if disruption of the urothelium were occurring.

Vesicular release of ATP is a Ca^{2+}-dependent process (19). Experiments were carried out in the absence of extracellular Ca^{2+} in the perfusion medium and equilibrated for two different periods of time. Perfusion in Ca^{2+}-free medium for 1 h before distension of the ureter did not inhibit distension-evoked ATP release; however, perfusion in Ca^{2+}-free medium for 2 h before distension caused a significant decrease in the amount of ATP released at the three increasing distension pressures that were tested. These results further support the hypothesis that the ATP is being released via vesicular exocytosis.

The mechanism by which ATP is released from nerve terminals has been shown to be vesicular discharge (47) and the action of ABC protein family members in some nonneuronal cells (42, 50). The mechanism governing the release of ATP from ureter epithelial cells in response to distension appears to involve vesicular exocytosis, and although the lack of activity of the inhibitors for ABC transporters would seem to rule out involvement in ATP release, there is some debate regarding the direct role of these ABC proteins in the transport of ATP across membranes (24, 31). There is a suggestion that these proteins may have an indirect action on ATP release, perhaps the facilitation of its release by other mechanisms (41).

The role of the ATP released from the urothelium after distension can as yet only be surmised, although there is strong evidence for a mechanosensory transduction mechanism; ATP released from the urothelium can act as a signal for distension and in turn for the pain associated with such distension (8, 9, 10). Receptors sensitive to ATP have been cloned from subpopulations of nociceptive sensory neurons in dorsal root, trigeminal, and nodose ganglia (7, 32, 53, 54) and are expressed on their central and peripheral axonal extensions. Recently, evidence for the presence of $P2X_3$ receptor immunoreactivity on occasional nerve bundles in the subepithelial plexus of the rat ureter (29) has been presented. These several pieces of evidence lend support to the recent hypothesis of Burnstock (9) concerning the role of ATP in mechanosensory transduction and nociception. Burnstock proposed that distension of structures such as blood vessels, ureters, urinary bladders, and lungs results in the release of ATP, which then acts on $P2X_{2/3}$ receptors on subepithelial sensory nerves to convey information to the central nervous system. Support of this is already available for the urinary bladder of rabbits (20, 37), rats (27, 39), and mice (15, 52).

In summary, this study has demonstrated that, upon distension, the guinea pig ureter releases ATP from the urothelium, and the release is not a result of damage to the epithelium. The mechanism of ATP release involves vesicular exocytosis, although the involvement of members of the ABC protein superfamily should not be discounted. The ATP that is released after distension is hypothesized to play a role in mechanosensory transduction by stimulation of nociceptive $P2X_3$ receptors. This hypothesis is currently under investigation.

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