Intraluminal ATP Concentrations in Rat Renal Tubules

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It is becoming increasingly recognized that stimulation of apical P2 receptors can influence solute transport in the nephron, but, to date, no information is available on endogenous intraluminal nucleotide concentrations in vivo. This study measured intraluminal ATP concentrations in the renal tubules of anesthetized rats. Proximal tubular concentrations were found to be in the range of 100 to 300 nmol/L, with no significant variation along the S2 segment, whereas concentrations in the early distal tubule were markedly lower. Using collections of varying duration, the half-life of ATP in collected proximal tubular fluid was found to be 3.4 min, indicating significant breakdown by soluble nucleotidases. For assessment of whether proximal tubular ATP was filtered or secreted, experiments were performed in Munich-Wistar rats. The ATP concentration in midproximal tubules (142 ± 23 nmol/L) was more than four-fold higher than in Bowman’s space (32 ± 7 nmol/L; P < 0.001), whereas fractional water reabsorption between the two sites was modest. In experiments that were designed to determine the effects of (patho)physiologic disturbances on intraluminal ATP, rats were either volume expanded or subjected to hypotensive hemorrhage. Neither maneuver affected proximal tubular luminal ATP concentrations significantly; rapid degradation of secreted ATP by ecto- and soluble nucleotidases is a possible explanation. It is concluded that the proximal tubule secretes ATP into the lumen, where it may have an autocrine/paracrine regulatory role.


The epithelial cells of the mammalian nephron express a variety of P2Y and P2X receptor subtypes on their basolateral and apical membranes (1–3). When stimulated by application of exogenous nucleotides, these P2 receptors can influence a number of transport processes. Thus, activation of basolateral P2 receptors in the collecting duct inhibits vasopressin-stimulated water reabsorption (4–6), stimulation of apical P2 receptors can affect solute transport in both the proximal and the distal nephron: a recent in vivo micropuncture study in the rat showed that stimulation of apical P2Y1 receptors inhibits proximal tubular bicarbonate reabsorption through suppression of NHE3 activity (7), and in vitro evidence indicates that stimulation of apical P2 receptors in the distal nephron inhibits amiloride-sensitive sodium reabsorption (8,9) and reduces the activity of apical K+ secretory channels (10). These findings clearly support the proposal that intraluminal nucleotides, acting on apical P2 receptors, can function as autocrine/paracrine regulators of tubular transport.

Before making such inferences concerning a functional role of intraluminal nucleotides, however, it is necessary to ascertain whether their normal endogenous concentrations are sufficient to activate P2 receptors and whether they can be influenced by physiologic/pathophysiologic maneuvers that are known to affect tubular transport. ATP secretion by renal epithelial cultures and by cell lines that were derived from specific nephron segments was reported previously by Schwiebert’s group (3,11). It was shown that cultured cells released measurable amounts of ATP into both apical and basolateral media, with apical release predominating. However, although in vitro studies are useful pointers, determinations of ATP concentrations in cell culture media clearly cannot provide information on ATP concentrations in the tubular lumen in vivo. To date, no such measurements in native, intact tubules have been reported. The purpose of our study, therefore, was to assess endogenous ATP concentrations within the lumen of proximal and distal tubules of the rat nephron and to determine whether the ATP was filtered or secreted. Tubular fluid samples were obtained using in vivo micropuncture techniques, and the samples were assayed for ATP using the luciferin/luciferase reaction.

Materials and Methods

Sprague-Dawley rats or Munich-Wistar rats were anesthetized with sodium thiopentone (100 mg/kg, intraperitoneally; Link Pharmaceuticals, Horsham, Sussex, UK) and prepared surgically for micropuncture of the left kidney (12). All animals received intravenous infusions of isotonic saline at 4 ml/h. After a 2-h equilibration period, micropuncture collections were begun. In some rats, [3H]inulin (40 μCi bolus, 40 μCi/h; Amersham Biosciences, Chalfont St. Giles, Bucks, UK) was infused intravenously from the end of the first hour of equilibration. Micropuncture collections, using sharpened glass micropipettes (tip diameter 8 to 11 μm) that were filled with Sudan black-stained oil, were made from Bowman’s space (Munich-Wistar rats only), proximal convoluted tubules, and, in some cases, early distal tubules, using methods described previously (13). Unless otherwise stated, collections lasted 4 min. Areas of kidney surface were covered in a systematic manner so that only previously unpunctured regions were selected for tubular fluid collections. This was done to prevent the same nephron’s being punctured more than once.
Munich-Wistar Rats

To assess the contribution of glomerular filtration to the levels of ATP measured in the proximal tubule, we compared, in the same animals, ATP concentrations in Bowman’s space and midproximal convoluted tubule in 12 Munich-Wistar rats (whose kidneys have multiple surface glomeruli accessible to micropuncture. Collections were made alternately from the two sites. Identification of Bowman’s space was made on the basis of its proximity to a surface glomerulus, its characteristic shape when partially filled with stained oil, and the large number of proximal tubular segments that were seen to succeed it when a small oil droplet was allowed to move downstream (14). Proximal tubular collections were made from segments that had four to five more surface convolutions beyond the collection site. In some animals (n = 4), the tubular fluid/plasma ratio measured at each collection site for determination of how much water was reabsorbed between the two sites. In these animals, three small arterial blood samples (approximately 80 μl) were taken at approximately similar time intervals for measurement of plasma [3H]insulin activity. At the end of the experiment, a larger blood sample was taken and the plasma was deproteinized to compare the [3H]insulin counts of plasma and plasma water, and an appropriate correction factor then was applied to the counts that were measured in the small plasma samples that were taken previously. For ATP determinations, all tubular collections from a given site (n = 6 to 10) in each rat were pooled in a vial that contained 50 μl of ice-cold deionized water. The vial was kept frozen between additions of fresh collections.

Sprague-Dawley Rats

Luminal ATP Concentrations in Early, Mid, and Late Proximal Convolutions. In nine rats, luminal ATP concentrations were compared in different regions of the S3 segment of the proximal convoluted tubule. The position of the collection pipette was classified according to the number of surface loops beyond the collection site: Six to seven more loops = “early”; three to four more loops = “mid”; zero to one more loop = “late.” Confirmation of the late proximal site was achieved by intratubular injection of silicone rubber solution (Microfil; Flow Tech, Carver, MA) and subsequent microdissection (15). Collections from each site (n = 7 to 10) were pooled as described above; thus, a single pooled value for each site was obtained per rat.

Distal Tubular ATP Concentrations. In nine rats, collections were made either from midproximal convoluted tubules or from early distal tubules. The early distal segments initially were identified after intravenous injection of Lissamine Green (30 μl of a 5% solution) and subsequently confirmed using intratubular Microfil injections. All of these collections lasted 8 min; as much as possible, they were made alternately from the two sites, although collections were made from a greater number of distal (up to 16) than proximal (up to 10) tubules. On two occasions, it was necessary to pool distal tubular collections from two rats; in these cases, the ATP concentrations of the pooled proximal collections from the two rats were averaged for comparison with the early distal ATP concentration.

Assessment of Possible Artifacts. To assess whether measured ATP concentrations were influenced by changes in intraluminal pressure caused by the presence of the oil column distal to the point of collection, in three rats we compared ATP concentrations in normal (pooled) collections with those in pooled collections for which no oil block was used. In an additional three rats, we compared ATP concentrations in normal (pooled) collections with those in pooled collections for which a 4-min delay was allowed to elapse before the oil block was deposited. To assess the degree of contamination of the pipette tip with intracellular ATP during the micropuncture procedure, on two occasions a pipette was inserted into 10 tubules but without tubular fluid collection. The pipette tip then was washed in 50 μl of deionized water, which then was assayed for ATP.

Degradation of ATP during Micropuncture Collection Procedures. The apical membrane of renal tubules contains a number of ectonucleotidases, some of which can be cleaved, releasing soluble nucleotidases that could degrade ATP. In pilot experiments, the stability of ATP in proximal tubular fluid was investigated by determination of the degradation of exogenous ATP. Fluid was collected from midproximal tubules, and aliquots (each 82 nl) were deposited under oil on a watch glass. Samples of standard ATP solution (12 nl of 100 μM ATP) were mixed with the tubular fluid samples and allowed to incubate at room temperature (22°C) for 5, 10, 20, or 40 min, at the end of which any degradation was halted by addition of the whole solution to 50 μl of ice-cold deionized water and freezing for subsequent assay. The same standard ATP solution was added (in triplicate) to deionized water as a control. Because these experiments indicated significant degradation of the (much lower levels of) endogenous ATP found in collected fluid. In nine rats, collections of varying duration were made from midproximal tubules. The collections lasted for 4, 10, or 22 min; the order in which they were made was randomized, and all samples of a given duration in each rat were pooled.

Effect of Volume Expansion or Hypotensive Hemorrhage on Proximal Tubular ATP Concentrations.

Time Controls. To check whether proximal tubular ATP concentrations altered during the time course of the experiment, we performed time controls. Midproximal collections were taken for 1 h in eight rats, the collections (n = 7 to 10) being pooled; then, after a 30-min delay, further collections were made for 1 h, the collections (n = 7 to 10) again being pooled (in a separate vial).

Volume Expansion. Because apical P2 receptor activation inhibits proximal tubular reabsorption (7), we tested the hypothesis that acute extracellular volume expansion, which leads to reduced fractional fluid reabsorption in the proximal tubule, might result in increased intraluminal ATP concentrations in the proximal tubule. In eight rats, collections were made from midproximal tubules for 1 h, and the collections (n = 7 to 10) were pooled. The rats then received intravenous infusions of isotonic saline at 24 ml/h. After 30 min, further midproximal collections were made during the ensuing hour, and the collections (n = 7 to 10) were pooled in a separate vial.

Hypotensive Hemorrhage. It has been proposed that ATP release into the lumen should increase under ischemic conditions, thereby helping to protect the tubular epithelium by inhibiting energy-consuming tubular transport processes (16). We therefore tested the hypothesis that the partial renal ischemia after hypotensive hemorrhage results in increased intraluminal ATP concentrations. In eight rats, collections were made from midproximal tubules for 1 h, and the collections (n = 7 to 10) were pooled. The rats then were bled (15 ml/kg body wt) from the femoral artery. Thirty minutes later, additional midproximal collections were made during the ensuing hour, and the collections (n = 7 to 10) were pooled in a separate vial.

Statistical Analyses

All micropuncture collections initially were deposited under oil, and their volumes were measured using calibrated constriction pipettes. Known volumes then were deposited in ice-cold deionized water and frozen (before ATP measurement), as described above (total pooled tubular fluid volume 700 to 1800 nl), and, in some cases, duplicate samples (79 nl for Bowman’s space, 38 nl for proximal tubule) were taken for measurement of [3H]insulin activity. The ATP concentration of pooled tubular fluid samples was measured using the luciferin/luciferase enzyme reaction. The samples (ap-
Approximately 51 μl were deposited in the wells of a nonphosphorescent microplate and processed automatically by injection of 100 μl of luciferin-luciferase reagent (ATP Reagent SL; Bio Thema, Handen, Sweden) into each well; the emitted light was quantified using a luminometer (Lucy 1; Anthos Labtec, Salzburg, Austria). An appropriate calibration curve was constructed using ATP standards.

[^3H]inulin activities in Bowman’s space, proximal tubular fluid, plasma, and plasma water were determined by β-scintillation counting (model 2900 TR; Canberra-Packard, Pangbourne, UK) after dispersal in Aquasol 2 scintillation fluid (Perkin Elmer Life Sciences, Cambridge, UK). Urinary sodium concentrations were measured using a flame photometer (model 543; Instrumentation Laboratory, Warrington, UK).

**Statistical Analyses**

Values for ATP concentration are presented as individual paired data for each rat and as means ± SEM. Mean values were compared using one-way ANOVA with repeated measures and paired t test, as appropriate.

**Results**

**ATP Concentrations in Bowman’s Space versus Midproximal Tubule**

In Munich-Wistar rats, mean arterial BP (MABP) was 128 ± 2 mmHg (mean ± SEM), with no statistically significant change during the course of the experiment. It was documented previously that this strain of rat has a higher BP than found in other strains (14,17). In all 12 Munich-Wistar rats, the ATP concentration was higher in the proximal tubule than in Bowman’s space (P < 0.001; Figure 1). The mean (± SEM) ATP concentration in midproximal convoluted tubule was 142 ± 23 nmol/L, compared with only 32 ± 7 nmol/L in Bowman’s space. In some rats, TF/P_in also was measured at each site. TF/P_in at Bowman’s space was 1.04 ± 0.02 (n = 14), and at the midproximal tubule it was 1.34 ± 0.06 (n = 14), indicating that approximately 25% of the filtered fluid had been reabsorbed between the two puncture sites.

**Figure 1.** ATP concentrations in Bowman’s space and midproximal convoluted tubule of Munich-Wistar rats. Each point represents a single pooled collection, made up of 6 to 10 samples. Values in each rat are linked by solid lines. Means ± SEM also are shown.

**Luminal ATP Concentrations along the Proximal Convoluted Tubule and in Early Distal Tubules**

In Sprague-Dawley rats that were not subjected to volume expansion or hemorrhage, MABP for the group as a whole was 108 ± 2 mmHg (n = 33), with no statistically significant change during the course of the experiment. Figure 2A shows ATP concentrations in early, mid, and late regions of the proximal convoluted tubule (as defined in Materials and Methods). No significant differences were found with respect to ATP concentration between the three sites. In contrast, in all seven comparisons between early distal tubule and midproximal tubule, the distal tubular ATP concentration (33 ± 14 nmol/L) was lower than the corresponding value in the proximal tubule (116 ± 33 nmol/L; P < 0.01; Figure 2B).

**Assessment of Possible Artifacts**

When midproximal tubular fluid merely was sampled (i.e., no oil block was used), ATP concentration was 147 ± 18 nmol/L (n = 3), compared with 104 ± 42 nmol/L (n = 3; NS, paired t test) when normal collections were made in the same rats. When a 4-min delay was allowed to elapse before insertion of an oil block, ATP concentration was 166 ± 47 nmol/L (n = 3), compared with 125 ± 15 nmol/L (n = 3; NS, paired t test) when normal collections were made in the same rats. On two occasions, a pipette was inserted into 10 tubules (without collecting tubular fluid), after which its

**Figure 2.** Intraluminal ATP concentrations along the proximal convoluted tubule and in early distal tubule. (A) ATP concentrations in early, mid, and late regions of the proximal convoluted tubule of Sprague-Dawley rats. Each point represents a single pooled collection, made up of 7 to 10 samples. Values in each rat are linked by solid lines. Means ± SEM are indicated. (B) ATP concentrations in midproximal convoluted tubule and early distal tubule of Sprague-Dawley rats. Each point represents a single pooled collection, made up of 7 to 10 proximal samples or 10 to 16 distal samples. Values in each rat (or pair of rats) are linked by solid lines. Means ± SEM also are shown.
Degradation of ATP during Micropuncture Collection Procedures

As indicated in Materials and Methods, it is possible that tubular fluid contains a number of soluble nucleotidases that could degrade ATP. There inevitably was a finite time between the start of a tubular fluid collection and the inhibition of any metabolic reactions by deposition of the sample in ice-cold water and freezing it. We therefore investigated the stability of ATP in the collected fluid. This was done first by incubating known amounts of ATP standards in samples of collected proximal tubular fluid and determining the rate of ATP degradation in vitro. Under these conditions, where substrate concentration was not a limiting factor, ATP was found to be degraded at a mean rate of 16 fmol/min (Figure 3A), which, if mimicked in vivo, would have a profound influence on measured values of endogenous ATP. We therefore assessed directly the degradation of endogenous ATP in collected proximal tubular fluid (at room temperature) plus 2 min (the time taken for volume measurement). "Processing time" was defined as half the total duration of collection (to obtain an average time for the existence of a droplet of tubular fluid in the pipette, assuming a constant collection rate) plus the 2 min taken for volume measurement. ATP concentration fell exponentially as processing time increased; its half-life was 3.4 min. Extrapolation back to zero time (i.e., before any degradation by soluble nucleotidases had occurred) gave a value of 275 nmol/L.

Effect of Volume Expansion or Hypotensive Hemorrhage

In time control experiments, MABP was 110 ± 4 mmHg during the first control period and 108 ± 4 mmHg during the second control period (NS). Although there was considerable variation in proximal tubular ATP concentration (in pooled samples) between rats, there was no systematic difference in ATP concentration between the two collection periods; indeed, values for the two periods were remarkably similar (Figure 4A).

In rats that were subjected to volume expansion, MABP was 109 ± 4 mmHg during the control period and 106 ± 4 mmHg during the experimental period (NS). Volume expansion caused predictable increases in sodium excretion (3.2 ± 0.6 μmol/min [micropuncture kidney] during the control period versus 14.2 ± 1.6 μmol/min during the experimental period; P < 0.001) and in midproximal tubular flow rate (30 ± 1 nl/min, control period versus 36 ± 2 nl/min, experimental period; P < 0.01). However, no significant change in proximal tubular ATP concentration was found (Figure 4B).

In rats that were subjected to hemorrhage (15 ml/kg), MABP was 112 ± 4 mmHg during the control period, fell to 42 ± 3 mmHg immediately after bleeding, and recovered partially to 92 ± 2 mmHg during the period 30 to 90 min after bleeding (experimental period). Sodium excretion during the experimental period was lower than during the control period (3.1 ± 0.5 μmol/min, control period versus 0.5 ± 0.2 μmol/min, experimental period; P < 0.01), as was midproximal tubular flow rate (34 ± 2 versus 17 ± 2 nl/min; P < 0.001). Although in two rats the posthemorrhage ATP concentration was strikingly higher than the prehemorrhage value (Figure 4C), taking the group as a whole, there was no statistically significant effect.

Discussion

ATP concentrations in the proximal tubular fluid, as measured in this study, are lower than those predicted on the basis of in vitro experiments with tubular cultures (3) but nevertheless are high enough to stimulate a number of P2 receptor subtypes (3,18). Moreover, the concentrations that were measured in 4-min collections underestimated those in the lumen at the tip of the pipette, owing to ATP degradation during the collection procedure. Assessment of nucleotide catabolism by incubation of an excess of exogenous ATP in collected proximal tubular fluid and determination of its rate of degradation (Vmax) indicated that tubular fluid contains enzymes that are capable of hydrolyzing ATP. Subsequently, the half-life of endogenous ATP in collected proximal tubular fluid (at room
temperature) was shown to be 3.4 min, and by extrapolation we were able to calculate that the average concentration of ATP in the tubular lumen before any degradation by soluble nucleotidases had occurred was approximately 275 nmol/L. *In vivo* (i.e., within the tubule at 37°C), this degradation of endogenous ATP by soluble nucleotidases would occur at a significantly greater rate. In addition, an array of ectonucleotidases is known to line the proximal tubular apical membrane (19–22). These ectonucleotidases are poised to metabolize ATP close to its site of action, even before tubular fluid collections can proceed. For this reason, we believe that the concentrations of ATP in the immediate vicinity of apical P2 receptors will be substantially higher than those that are measurable with this method.

In any study of this nature, care must be taken to exclude possible artifacts and potential sources of contamination. It could be argued, for example, that disturbances of intraluminal pressure caused by the oil column distal to the point of collection might induce mechanical stress and stimulate ATP release by the proximal tubular cells, or that damage to the tubular cells caused by insertion of the collection pipette might itself lead to release of intracellular ATP into the lumen. More fundamental, contamination of the pipette tip by ATP as the pipette traverses the tubular cells also might lead to artificial intraluminal ATP concentrations (intracellular ATP concentrations being several orders of magnitude greater than those measured in the lumen). These possibilities all were assessed in our study. We found no difference in luminal ATP concentrations regardless of whether we used an oil block or whether a 4-min delay was interposed between insertion of the pipette and deposition of the oil block. Clearly, any pulse of ATP released on insertion of the pipette must have been washed away/metabolized within seconds, before tubular fluid collections began. Finally, we performed some experiments in which a pipette was inserted repeatedly into a number of tubules but without tubular fluid collection and the pipette tip then was washed in deionized water. No trace of ATP could be detected. We conclude that the ATP that was measured in the proximal tubular lumen was not simply a consequence of the experimental manipulations used.

The next question to be addressed was the source of this luminal ATP. Is it merely filtered at the glomerulus, or is it secreted by the proximal tubular cells? Our experiments in Munich-Wistar rats showed that the ATP concentration in midproximal convoluted tubule of Sprague-Dawley rats during a 1-h control period (control 1) and during the period 30 to 90 min later (control 2). (B) Volume expansion: ATP concentrations in midproximal convoluted tubule of Sprague-Dawley rats during a 1-h control period and during the period 30 to 90 min after the start of an intravenous saline infusion of 24 ml/min (“volume expansion”). (C) Hypotensive hemorrhage: ATP concentrations in midproximal convoluted tubule of Sprague-Dawley rats during a 1-h control period and during the period 30 to 90 min after a blood loss of 15 ml/kg (“hemorrhage”). In all three groups of rats, each point represents a single pooled collection, made up of 7 to 10 samples. Values in each rat are linked by solid lines. Means ± SEM also are shown.
The mechanism of ATP secretion by the tubular cells is unknown. There is considerable evidence from nonrenal epithelia for vesicular release/exocytosis of ATP (23,24); other possible routes include maxi-anion channels (25) or connexin hemichannels (26). It is possible that both vesicular and nonvesicular mechanisms of ATP release can occur, depending on cell type and/or the specific stimulus involved. Recently, in vitro experiments in our laboratory provided some support for the notion of vesicular release of ATP in the proximal tubule. Using quinacrine as a marker, intracellular ATP-containing vesicles were identified in a proximal tubular S1 cell line. Exposure of the cells to hypotonic shock led to a reduction in intracellular fluorescence, coupled with a marked increase in ATP concentration in the medium (27). Although caution should be exercised in interpreting the results of an experiment involving an immortalized cell line and a nonphysiologic stimulus, the observation at least supports the principle of exocytotic ATP release.

Our measurements of ATP concentrations in the distal tubule, demonstrating that they are markedly lower than those in the proximal tubule, echo the in vitro findings of Schwiebert’s group in cultured cells (3) and suggest that an effect of P2 receptor stimulation in the distal tubule under physiologic conditions is less likely. However, in view of the longer collection times used and the presence of some ectonucleotidases in this nephron segment (22), such an effect cannot be ruled out. Parenthetically, that lower ATP concentrations were measured in the distal tubule (and in Bowman’s space) is further circumstantial evidence that our determinations in the proximal tubule were not simply the consequence of injury-induced release of ATP.

Finally, our studies attempted to address the question of whether intratubular ATP concentrations are altered by (patho)physiologic maneuvers that affect tubular reabsorptive processes. Given the inhibitory effects of intraluminal nucleotides on tubular reabsorption (see the beginning of this article), it could be speculated that the reduction in fractional proximal reabsorption that accompanies acute volume expansion might result partly from enhanced ATP secretion. However, despite a marked natriuresis and significant increase in midproximal tubular flow rate, volume expansion had no systematic effect on measured intratubular ATP concentrations. Another situation in which ATP secretion might be expected to increase is renal ischemia. There is evidence that ischemia induces ATP release in nonrenal tissues (25,28), and it has been proposed that intraluminal ATP might serve to protect the renal tubular epithelium under ischemic conditions by inhibiting energy-consuming transport processes (16). Moreover, we have shown previously that the partial renal ischemia that follows hemorrhage has little effect on fractional proximal tubular reabsorption (29) despite enhanced activity of the renal sympathetic nerves and the renin-angiotensin system; it seems that the stimulatory effects of these systems are offset by an inhibitory effect of unknown source. In the event, although hypotensive hemorrhage seemed to cause a marked increase in intratubular ATP concentration in two animals (contrasting with the stability seen in time controls), in the group as a whole, there was no statistically significant change.

Although at first glance these negative findings suggest that proximal tubular ATP release is “constitutive,” lacking physiologic control, there is some evidence that such a view may be oversimplistic. As indicated earlier, the proximal tubular brush border membrane contains a number of ectonucleotidases that are capable of metabolizing ATP, and it may be relevant that, in astrocytes at least, the sites of ATP release and of ectonucleotidase activity are co-localized (30). Moreover, it has been shown that sympathetic stimulation of guinea pig vas deferens evokes the simultaneous release of both ATP and soluble nucleotidases (31), and a similar phenomenon has been described when vascular endothelial cells are exposed to shear stress (32). Therefore, it is feasible that enhanced ATP release in our experiments could have been masked by its immediate partial degradation by ecto- and soluble nucleotidases. In this context, it should be noted that the inhibitory effects of apically applied nucleotide on proximal tubular NHE3 activity (7) and Na+/K+ATPase (33) are mediated by the P2Y1 receptor subtype, which has a much greater sensitivity to ADP than to ATP (18). It would make physiologic sense, therefore, for locally released ATP to be converted rapidly to its diphosphate form.

After volume expansion or hemorrhage, there did seem to be a greater variation in intratubular ATP concentration than seen either in the same rats before the maneuver or in time controls (see Figure 4). Although we do not have a ready explanation for this observation, it seems possible that the multifactorial physiologic changes that are associated with volume expansion or hemorrhage may have varied from rat to rat so that tubular ATP secretion was affected to differing extents between rats. Another theoretical possibility is that ATP concentrations in glomerular plasma and, therefore, in glomerular filtrate varied considerably between rats. This possibility was assessed in a separate group of Munich-Wistar rats (n = 6) that were subjected to hemorrhage. Similar results were obtained to those seen in Sprague-Dawley rats (i.e., no significant hemorrhage-induced change in proximal tubular ATP concentration, with a wide spread of posthemorrhage values). However, there was no correlation between ATP concentrations in Bowman’s space and proximal tubules; indeed, in only one animal did the ATP concentration in Bowman’s space alter after hemorrhage.

Conclusion
Physiologically significant concentrations of ATP are present in the lumen of the proximal tubule, and the evidence strongly suggests that the nucleotide is secreted/released by the epithelial cells themselves. Release of ATP into the lumen and the presence of apical P2 receptors as well as of enzymes that are capable of controlled nucleotide degradation, strengthen support for the proposition that extracellular nucleotides can act as important autocrine/paracrine regulators of tubular function.

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References


