P2 purinoceptor localization along rat nephron and evidence suggesting existence of subtypes P2Y₁ and P2Y₂

SEOK HO CHA, TAKASHI SEKINE, AND HITOSHI ENDOU
Department of Pharmacology and Toxicology,
Kyorin University School of Medicine, Mitaka, Tokyo 181, Japan

Cha, Seok Ho, Takashi Sekine, and Hitoshi Endou. P2 purinoceptor localization along rat nephron and evidence suggesting existence of subtypes P2Y₁ and P2Y₂. Am. J. Physiol. 274 (Renal Physiol. 43): F1006–F1014, 1998.—Effects of extracellular ATP on intracellular free calcium concentration ([Ca²⁺]; i) were examined in rat single nephron segments using the fura-2 AM. ATP (10 µM) induced a significant transient increase in [Ca²⁺]i in the glomerulus, the early proximal convoluted tubule (S1), the cortical collecting tubule (CCT), and the outer medullary collecting tubule (OMCT). The magnitude of the response was the greatest in the OMCT among four segments. ATP induced an increase in [Ca²⁺]i in a dose-dependent manner in S1 and OMCT. In the OMCT, ATP caused a biphasic increase in [Ca²⁺]i consisting of an initial rapid rise and a sustained phase. Removal of calcium from the medium resulted in an attenuation of the sustained phase of [Ca²⁺]i and an ~30% reduction in the height of the initial [Ca²⁺]i peak in response to 10 µM ATP. Effects of ATP, its analogs, and its metabolites were tested in the S1 and OMCT. ATP, 2-methylthio-ATP (2-MeS-ATP), ADP, and UTP increased [Ca²⁺]i dose dependently. AMP and adenosine did not affect [Ca²⁺]i in the S1 and OMCT. The ATP- or 2-MeS-ATP-induced [Ca²⁺]i increase was inhibited by the pretreatment of the S1 and OMCT with suramin or reactive blue 2. Neomycin, a phospholipase C inhibitor, attenuated the ATP-induced [Ca²⁺]i increase. To investigate the hormone-like action of ATP in OMCT, a heterologous cross desensitization was performed. The pretreatment of OMCT with ATP inhibited increases in vasopressin-, ANG II-, endothelin-1-, or bradykinin-induced [Ca²⁺]i increase. These findings suggest that ATP might affect the above peptidyl agonist-activated calcium mobilizations.

Adenosine 5’-triphosphate; intracellular free calcium; early proximal convoluted tubule; outer medullary collecting tubule

**POTENT ACTIONS OF PURINE NUCLEOTIDES AND NUCLEOSIDES**

were first reported over 60 years ago by Drury and Szent-Györgyi (7). The role of intracellular ATP as a source of energy, a phosphate group donor in phosphorylation reactions, and a substrate of ATPases and adenylyl cyclase was established many years ago (9). Cells possess complex mechanisms for the synthesis of ATP and for its control at the cytosolic level. ATP can be released during neurotransmission into the synaptic space or from "dense granules" during blood platelet activation (24), and stimulation with BA²⁺ and high K⁺ concentration causes the release of adenine nucleotides from a cultured sympathetic neuron (20). ATP and norepinephrine are co-released from an isolated rat kidney upon renal nerve stimulation (2). In addition, cell damage during tissue injury (e.g., vascular rupture) may also lead to the release of ATP into the extracellular space. Such is the case in the thymus, where lymphocyte differentiation induces extended proliferation and cell death, which may lead to very high ATP concentrations, particularly in the cortex and the corticomedullary junction (13). Therefore, based on the above sources, it appears that extracellular ATP concentrations can attain active levels for extracellular ATP receptor stimulation. The local ATP content will depend on the amount released, the effect of dilution, and the efficiency of adjacent ectonucleotidases. Ectonucleotidases are responsible for extracellular ATP hydrolysis (29); three different enzymes (ecto-ATPase, ecto-ADPase, and ecto-5′-nucleotidase) sequentially dephosphorylate ATP to adenosine. These ectoenzymes may play a role in the modulation of extracellular ATP-induced functional changes. It is well known that extracellular ATP mediates various physiological actions via specific receptors on the surface of cells, termed purinergic receptors or purinoceptors. In 1990, Burnstock (4) proposed a basis for discriminating the two types of purinergic receptor, which was based largely on an analysis of information contained in many previous reports regarding the actions of purine nucleotides and nucleosides on a wide variety of tissues (4). Adenosine and AMP have a higher affinity for the P1 purinoceptor than do ATP and ADP. In contrast, ATP and ADP have a higher affinity for the P2 purinoceptor than do AMP and adenosine. The P1 purinoceptors were subdivided into A₁/R₁ and A₂/R₂ subtypes according to their relative affinity for a series of adenine analogs and whether adenosine cyclase activity is increased (A₂/R₂) or decreased (A₁/R₁) in the presence of adenosine (4).

P2 purinoceptors have been subdivided into P2X and P2Y subtypes on the basis of their relative affinity for synthetic ATP and ADP derivatives (16). There are many receptor subtypes, which are P2X₁ through P2X₇, and P2Y₁ through P2Y₁₂ (25).

Regarding the kidney, much less is known concerning the pharmacological and physiological events involved in P2 purinoceptor action. However, it has recently been demonstrated that P2 purinoceptors stimulate the formation of inositol 1,4,5-trisphosphate (IP₃) in the rat renal cortex (23), release arachidonic acid and metabolites in MDCK cells (11), mediate an increase in intracellular free calcium concentration ([Ca²⁺]i) in the LLC-PK₁, and cultured glomerular endothelial and mesangial cells (3, 15, 27, 28), and mediate the regulation of adenosine cyclase and protein kinase C activity (1). However, the localization of ATP receptors and the determination of their functional activity in specific nephron segments has not been carried out satisfactorily to date.
We investigated the effects of ATP on [Ca\(^{2+}\)]\(_i\) in freshly isolated nephron segments from rat kidneys and attempted to evaluate the mechanisms by which ATP alters [Ca\(^{2+}\)]\(_i\) in the renal tubule segments. Several types of purinoceptor agonists and antagonists were used for the identification of ATP-responsive purinoceptor subtypes.

**MATERIALS AND METHODS**

Animals. Male Sprague-Dawley rats weighing 200–240 g were purchased from Saitama Experimental Laboratories (Saitama, J. apan) and housed in commercial equipment in a conventional environment for at least 3 days prior to use. The rats were provided with a pelleted standard diet (Oriental Yeast, Osaka, J. apan) and tap water ad libitum.

Materials. The materials used were purchased from the following sources: fura 2-AM was from Dojin Laboratories (Kumamoto, J. apan); ATP, ADP, and UTP were from Boeh- ringer (Mannheim, Germany); 2[\(^{35}\)P]-ATP and a[\(^{32}\)P]-Me-ATP were from Research Biochemicals (Natick, MA); AMP, adenosine, bovine serum albumin (BSA, fraction V), neomycin, [Arg\(^8\)]vasopressin, bradykinin (BK), reactive blue 2 (RB-2), ionomycin, EGTA, and collagenase were from Sigma Chemical (St. Louis, MO); fetal calf serum (FCS) was from Life Technologies (Bethesda, MD); suramin was from Wako (To- kyo, Japan); and holding solutions containing 10 µM fura 2-AM, 2 mM pyruvate, 2 mM aspartate, 2 mM glutamate, 10% FCS, and 0.5 mM CaCl\(_2\). To clarify the P2 purinoceptor distribution in isolated nephron segments, the [Ca\(^{2+}\)]\(_i\) was determined. As shown in Fig. 1, ATP at 10\(^{-5}\) M caused a significant and immediate increase in [Ca\(^{2+}\)]\(_i\) in isolated rat glomerulus, S1, CCT, and OMCT. The peak values of ATP-induced [Ca\(^{2+}\)]\(_i\) increases were as follows (as % of their basal levels): glomerulus, 147 ± 12%; S1, 139 ± 9%; CCT, 150 ± 16%; OMCT, 275 ± 16%. In contrast, ATP had no appreciable effects on the [Ca\(^{2+}\)]\(_i\) of the proximal straight tubules, the medullary thick ascending limb of Henle's loop, the cortical thick ascending limb of Henle's loop, and distal convoluted tubules (Table 1). UTP (10\(^{-5}\) M)-induced [Ca\(^{2+}\)]\(_i\) increases were observed in glomerulus, CCT, and OMCT (Table 1). Changes in fura 2 fluorescence showed a transient increase to a peak between 5–15 s after adding agonists and decreased until a steady level was reached between 90–120 s after the peak in the glomerulus and S1. In contrast, sustained increases in the [Ca\(^{2+}\)]\(_i\) in the CCT and OMCT were maintained for longer than 20 min following the application of ATP.
Fig. 1. Representative tracings of ATP-induced increases in intracellular free calcium concentration ([Ca\textsuperscript{2+}]) in isolated rat glomerulus (Glm, A), early proximal convoluted tubule (S1, B), cortical collecting tubule (CCT, C), and outer medullary collecting tubule (OMCT, D). ATP was applied as a bolus (arrowheads) at a final concentration of 10^{-5} M.

Table 1. Effects of ATP and UTP on [Ca\textsuperscript{2+}], of isolated single nephron segments

<table>
<thead>
<tr>
<th>Segment</th>
<th>Basal\textsuperscript{a}</th>
<th>Peak\textsuperscript{a}</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glm</td>
<td>59 ± 6</td>
<td>70 ± 8</td>
<td>&lt;0.01, &lt;0.05</td>
</tr>
<tr>
<td>S1</td>
<td>135 ± 9</td>
<td>94 ± 10</td>
<td>&lt;0.01, NS</td>
</tr>
<tr>
<td>S2</td>
<td>112 ± 5</td>
<td>92 ± 16</td>
<td>NS, NS</td>
</tr>
<tr>
<td>S3</td>
<td>93 ± 16</td>
<td>92 ± 16</td>
<td>NS, NS</td>
</tr>
<tr>
<td>MTAL</td>
<td>72 ± 6</td>
<td>74 ± 8</td>
<td>NS, NS</td>
</tr>
<tr>
<td>CTAL</td>
<td>107 ± 16</td>
<td>107 ± 16</td>
<td>NS, NS</td>
</tr>
<tr>
<td>DCT</td>
<td>102 ± 10</td>
<td>102 ± 10</td>
<td>NS, NS</td>
</tr>
<tr>
<td>CCT</td>
<td>134 ± 5</td>
<td>97 ± 10</td>
<td>&lt;0.01, &lt;0.02</td>
</tr>
<tr>
<td>OMCT</td>
<td>181 ± 8</td>
<td>97 ± 10</td>
<td>&lt;0.01, &lt;0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE for 4-6 animals. The time the peak appeared was always between 5-15 s after ATP or UTP addition. [Ca\textsuperscript{2+}], intracellular free calcium concentration; Glm, glomerulus; S1, proximal convoluted tubule; S2, second portion of the proximal tubule; S3, late proximal tubule; MTAL, medullary portion of thick ascending limb of Henle; CTAL, cortical portion of thick ascending limb of Henle; DCT, distal convoluted tubule; CCT, cortical collecting tubule; OMCT, outer medullary collecting tubule; NS, not significant.

Fig. 2. A: typical signalings of ATP-induced [Ca\textsuperscript{2+}] increases in S1 and OMCT. ATP was applied as a bolus (arrowheads) at the stated agonist concentrations. B: dose-response curves of peak values in [Ca\textsuperscript{2+}] of ATP-induced increases evoked transiently as a function of ATP concentrations in isolated rat S1 and OMCT. Data were obtained from experiments consisting of at least 6 experiments performed on different days. Values are means ± SE for 6 experiments. ATP-induced sustained increase in [Ca\textsuperset M] was prevented when S1 and OMCT were incubated in the Hanks' solution containing 1 mM EGTA but not when CaCl\textsubscript{2} 90 s before ATP addition. ATP-mediated (10^{-6} and 10^{-5} M for S1 and OMCT, respectively) transient [Ca\textsuperscript{2+}] increases could also be observed. The peak heights, however, were slightly attenuated (in S1: control, 182 ± 13%; Ca\textsuperscript{2+}, free, 145 ± 6%; and in OMCT: control, 285 ± 19%; Ca\textsuperscript{2+}, free, 225 ± 14%; respectively). Moreover, the ATP-induced sustained increase in [Ca\textsuperscript{2+}] disappeared due to the pretreatment of the OMCT with EGTA.
Effects of extracellular ATP, ATP metabolites, ATP analogs, and UTP on 
\([\text{Ca}^{2+}]_{i}\) increases.

In this series of experiments, we measured \([\text{Ca}^{2+}]_{i}\) in both the S1 and OMCT using ATP, ADP, AMP, adenosine, UTP, 2-\text{MeS-ATP} (P2Y-selective agonist), and \(\alpha,\beta\)-\text{Me-ATP} (P2X-selective agonist) for the characterization of P2 purinoceptor subtype. ATP, ADP, UTP, and 2-\text{MeS-ATP} induced increases in these \([\text{Ca}^{2+}]_{i}\) values in a dose-dependent manner, but AMP and adenosine failed to induce an increase in \([\text{Ca}^{2+}]_{i}\) in both of these nephron segments (Fig. 4, A and B). In contrast, an \(\alpha,\beta\)-\text{Me-ATP}-induced \([\text{Ca}^{2+}]_{i}\) increase was detected only at \(10^{-5}\) M (170 ± 9\% in S1 and 223 ± 19\% in OMCT). Although in OMCT, the average of maximal response to UTP was larger than that to ATP, it was not significantly larger. Similarly, the average \([\text{Ca}^{2+}]_{i}\) increases induced by ATP, UTP, and ADP were larger than that induced by 2-\text{MeS-ATP}. Concentrations of ATP higher than \(10^{-3}\) M could not be used because of fluorescent disturbance. The EC\(_{50}\) values in OMCT were estimated to be 6.2 × \(10^{-7}\) M for 2-\text{MeS-ATP}, \(4 \times 10^{-6}\) M for ATP, \(5 \times 10^{-6}\) M for ADP, and \(1.5 \times 10^{-5}\) M for UTP.

Effects of suramin or RB-2 on ATP-induced \([\text{Ca}^{2+}]_{i}\) change. To further characterize the receptor subtype mediating ATP responses in isolated rat S1 and OMCT, we used a selective P2Y agonist, 2-\text{MeS-ATP}, and the putative P2Y receptor antagonists, suramin and RB-2. As shown in Fig. 5, A and B, the pretreatment of both these nephron segments with suramin or RB-2 for 2 min dose-dependently inhibited ATP- or 2-\text{MeS-ATP}-induced \((10^{-5}\) M in S1 and \(5 \times 10^{-6}\) M in OMCT) \([\text{Ca}^{2+}]_{i}\) increases significantly.

Effects of extracellular ATP, ATP metabolites, ATP analogs, and UTP on IP\(_3\) production.

ATP, ATP metabolites, ATP analogs, and UTP were tested for their effects on IP\(_3\) production in cortical (concentration of agonist = \(10^{-3}\) M) and medullary (concentration of agonist = \(10^{-4}\) M) tubule suspensions. ATP stimulated IP\(_3\) formation at 252 ± 38\% and 327 ± 62\% of control levels within 20 s in cortical and medullary tubule suspension, respectively. The IP\(_3\) concentrations in the controls were 1.6 ± 0.3 and 0.8 ± 0.2 pmol/mg protein for cortical and medullary tubule suspensions, respectively. Figure 6 shows the IP\(_3\) production induced by ATP, ATP metabolites, ATP analogs, and UTP. The ratio of IP\(_3\) production

Fig. 3. A: representative tracings of ATP-induced \([\text{Ca}^{2+}]_{i}\) increases under conditions where the medium contained no calcium in isolated rat S1 and OMCT. To confirm the extracellular \text{Ca}^{2+}-free conditions, 1 mM EGTA was added to the media not containing CaCl\(_2\) 90 s before addition of ATP. B: effects of calcium removal from the medium on peak heights of ATP-induced \([\text{Ca}^{2+}]_{i}\) transients in isolated rat S1 and OMCT. Each column represents the relative change in \([\text{Ca}^{2+}]_{i}\) with respect to the basal \([\text{Ca}^{2+}]_{i}\) in each sample. Values are means ± SE for 4–7 experiments. TMB-8, 8-(N,N-diethylamino)octyl-3,4,5-trimethoxybenzoate hydrochloride. *P < 0.05 (vs. ATP alone). **P < 0.01.

Fig. 4. Dose-response curves of \([\text{Ca}^{2+}]_{i}\) increase induced by nucleotides, in isolated rat S1 (A) and OMCT (B). Various agonists were added to the medium after recording of basal values of \([\text{Ca}^{2+}]_{i}\). Ordinate represents the relative changes in calculated \([\text{Ca}^{2+}]_{i}\) with respect to the basal \([\text{Ca}^{2+}]_{i}\) before the addition of agonists to the medium. Basal \([\text{Ca}^{2+}]_{i}\) values of S1 and OMCT were 98 ± 6 and 69 ± 7 nM, respectively. Values are means ± SE for 5–9 experiments.
was significantly increased by the treatment of these tubule suspensions with ATP, ADP, UTP, or 2-MeS-ATP. Neither AMP, adenosine, nor $\alpha$, $\beta$-Me-ATP induced IP$_3$ production. Their efficacies with respect to IP$_3$ formation were similar to their efficacies with respect to inducing transient [Ca$^{2+}$]$_i$ increases.

Inhibitory effect of neomycin on ATP-induced [Ca$^{2+}$]$_i$ increase and IP$_3$ production. To evaluate the possible involvement of phospholipase C (PLC) in ATP-induced transient [Ca$^{2+}$]$_i$ increases and IP$_3$ production, the effect of neomycin was investigated. The isolated rat S1 and OMCT and cortical and medullary tubule suspensions were pretreated for 15 min with neomycin (3 x 10$^{-4}$ M), a PLC inhibitor, before the addition of ATP. As shown in Figs. 7 and 8, ATP-induced transient [Ca$^{2+}$]$_i$ increases and IP$_3$ production were significantly inhibited by neomycin.

Desensitization of ATP- and vasoactive peptidyl hormone-induced [Ca$^{2+}$]$_i$ increases in isolated rat OMCT. Vasoactive peptidyl hormones such as vasopressin (AVP), ANG II, ET-1, and BK also caused [Ca$^{2+}$]$_i$ increases in isolated rat OMCT (Fig. 9A). There was no heterologous cross desensitization between the peptidyl hormones used. Thus we compared the effects of ATP on [Ca$^{2+}$]$_i$ with those of vasoactive peptidyl hormones in isolated rat OMCT. Peptidyl hormones at a maximal dose (10$^{-6}$ M) evoked [Ca$^{2+}$]$_i$ transiently (Table...
2). The subsequent addition of the same peptidyl hormones caused no rises in \([\text{Ca}^{2+}]_i\), suggesting the presence of homologous desensitization for each transient \([\text{Ca}^{2+}]_i\) increase by the same agonist (data not shown). The successive addition of ATP to the medium after pretreatment of the OMCT with the above hormones induced a \([\text{Ca}^{2+}]_i\) rise of almost the same magnitude as that of the \([\text{Ca}^{2+}]_i\) rise induced by a single addition of ATP (Fig. 9B; Table 2). Next, to determine whether ATP induced a depletion of the \([\text{Ca}^{2+}]_i\) pool, heterologous cross desensitization and the effects of ionomycin on \([\text{Ca}^{2+}]_i\) were investigated under the calcium-free condition. Even under the calcium-free conditions, the peptidyl hormone-induced \([\text{Ca}^{2+}]_i\) increase was inhibited by the pretreatment of the OMCT with ATP. After the cross desensitization, ionomycin (10^{-5} \text{M}) increased \([\text{Ca}^{2+}]_i\) transiently, and this ionomycin-induced \([\text{Ca}^{2+}]_i\) increase was much larger than that induced by ATP in extracellular calcium-free conditions (Fig. 9C).

**DISCUSSION**

Previous studies have suggested the existence of both P1 and P2 purinoceptors in the kidney (23). The presence of ATP-responsive (P2) receptor subtypes in cortical slices (23), tubule suspension (5), cultured cells of renal tubular origin (1, 3, 15, 27) and isolated inner medullary collecting ducts (8) has been demonstrated.

<table>
<thead>
<tr>
<th>Agonists</th>
<th>% of Basal [Ca^{2+}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>Second</td>
</tr>
<tr>
<td>AVP</td>
<td>ATP</td>
</tr>
<tr>
<td>ANG II</td>
<td>ATP</td>
</tr>
<tr>
<td>ET-1</td>
<td>ATP</td>
</tr>
<tr>
<td>BK</td>
<td>ATP</td>
</tr>
<tr>
<td>ATP</td>
<td>AVP</td>
</tr>
<tr>
<td>ATP</td>
<td>ANG II</td>
</tr>
<tr>
<td>ATP</td>
<td>ET-1</td>
</tr>
<tr>
<td>ATP</td>
<td>BK</td>
</tr>
</tbody>
</table>

Values are means ± SE. Relative changes in \([\text{Ca}^{2+}]_i\) were calculated with respect to basal \([\text{Ca}^{2+}]_i\) in the same samples. Second agonist was added to the OMCT 5–7 min following the addition of the first agonist. Basal \([\text{Ca}^{2+}]_i\) of OMCT was 65 ± 9 nM. AVP, vasopressin; ET-1, endothelin-1; BK, bradykinin; NC, no change. AVP, ANG II, ET-1, and BK were 10^{-6} \text{M}; ATP was 10^{-4} \text{M}. **Fig. 8.** Effects of neomycin on ATP-induced IP_3 production in rat tubule suspensions. Neomycin was pretreated for 15 min before ATP addition. Control IP_3 contents of cortical and medullary tubule suspension were 1.4 ± 0.4 and 0.9 ± 0.3 pmol/mg protein, respectively. Values are means ± SE for 4 experiments. *P < 0.05 (vs. respective control).
However, localization of ATP receptors and the identification of their functional significance in specific tubule segments have not been carried out to date.

Because it is well known that ATP induces \([Ca^{2+}]_i\), mobilization (3, 8, 15), our present study provides several lines of evidence for the presence of ATP-responsive receptor subtypes, primarily in the S1 and OMCT, using the fluorescent probe fura 2. At first, we demonstrated that ATP receptors are present predominantly in the OMCT and to a lesser extent in the CCT, glomerulus, and S1 of isolated nephron segments in the rat. And UTP-induced \([Ca^{2+}]_i\) increases were observed in the glomerulus, CCT, and OMCT. Similar to the response of OMCT to ATP, the response of isolated nephron segments to UTP was the largest in the OMCT. The minimally \([Ca^{2+}]_i\)-increasing effective concentrations of ATP were between 10\(^{-6}\) and 10\(^{-5}\) M in the S1 and 10\(^{-8}\) and 10\(^{-7}\) M in the OMCT. Although plasma ATP concentrations may vary, these concentrations of ATP ranging from 10\(^{-8}\) to 10\(^{-5}\) M are likely to exist under physiological and/or pathological conditions. Recently, Ecelbarger et al. (8) have reported the existence of nucleotide receptors in the rat terminal collecting duct. They demonstrated that P2U subtypes are not sensitive to pertussis toxin. In other tissues, ATP other nucleotides, such as UTP, has also been described. Studies using other tissues have shown that \(\alpha,\beta\)-Me-ATP is 10 times more potent than ATP in stimulating the P2X purinoceptor and is equally or less potent in stimulating the P2Y purinoceptor. Based on this information, our findings clearly exclude P2T as a possible receptor in the rat S1 and OMCT. In the present experiments, ATP, UTP, \(\alpha,\beta\)-Me-ATP (P2X-specific agonist), and 2-MeS-ATP (P2Y-specific agonist) were compared with respect to their ability to induce \([Ca^{2+}]_i\) increases to clarify the receptor subtype(s) in the S1 and OMCT. The order of potency with respect to \([Ca^{2+}]_i\] increases was 2-MeS-ATP > ATP > ADP > UTP in the S1 and OMCT. However, 2-MeS-ATP at a maximal dose (above 10\(^{-4}\) M) was much less effective than ATP, ADP, and UTP in the OMCT (Fig. 4B). A similarly low apparent efficacy of 2-MeS-ATP has been reported for IP\(_3\) formation in endothelial cells (27). According to the data, the ATP-mediated receptor to induce \([Ca^{2+}]_i\) increases in the S1 should be the P2Y\(_1\) purinoceptor, and that in the OMCT may be the P2Y\(_1\) and P2Y\(_2\) purinoceptors. This conclusion is supported by the results of another experiment in which the antagonistic effects of suramin and RB-2, an anthraquinone-sulfonic acid derivative (17, 31), were investigated, and these compounds were found to inhibit ATP-induced transient \([Ca^{2+}]_i\) increases dose dependently. Therefore, the possibility that the P2Y\(_2\) purinoceptor exists in the OMCT can be considered, because the dose-response curves of ATP and UTP were different, and 2-MeS-ATP could not fully inhibit the response to ATP and UTP (data not shown).

Our present study may support the 1995 report of Zegarra-Morán et al. (33) that two different purinoceptors (P2Y and P2U) are present in the apical membrane of canine kidney cells.

Recently, Valera et al. (30) have reported that molecular cloning data on a new class of ligand-gated ion channel could define the P2X receptor for extracellular ATP, and several molecular cloning investigations have revealed that P2U, P2Y, and P2Y\(_1\) purinoceptors (from
mouse neuroblastoma cells, mouse and rat insulinoma, and the embryonic chick whole brain cDNA library by hybridization screening, respectively) are coupled with G proteins that have seven transmembrane-spanning domains (22, 28, 31). In these reports, the orders of agonist potencies for P2U and P2Y1 purinoceptors are given as ATP = UTP > ATPγS > 2-MeS-ATP = ADP > adenosine and 2-MeS-ATP ≥ ATP > ADP >> α,β-Me-ATP = UTP, respectively. These results indicate that molecular cloning studies are essential for the new classification of P2 purinoceptor subtypes.

The kidney is an important organ for controlling both the volume of body fluids and electrolytic balance. The distribution of renal gluconeogenic activities along the nephron has been determined (32). Yamada et al. (32) have reported that only the proximal tubules, mainly the S1 segments, had gluconeogenic ability. Glucose formed in the cortical tubule suspensions, therefore, should originate from the S1 segments. To elucidate the possible physiological significance of P2 purinoceptors, we demonstrated that gluconeogenesis in cortical tubule suspensions was stimulated by extracellular ATP and that this ATP-stimulated gluconeogenesis was inhibited by the treatment of the tubule suspensions with suramin (5). Because renal gluconeogenesis is localized exclusively in S1 (32), the regulation of gluconeogenesis is one of the functions of P2Y purinoceptors in the kidney.

In the signal transduction pathway in the kidney, vasoactive peptidyl hormones are very important. Therefore, the relationship between \([\text{Ca}^{2+}]\) induced by ATP and that induced by vasoactive peptidyl hormones was investigated. The ATP (10^{-4} M)-induced \([\text{Ca}^{2+}]\) increase was not attenuated by the pretreatment of the OMCT with a maximal concentration (10^{-6} M) of vasoactive peptidyl hormones, but the \([\text{Ca}^{2+}]\) increase induced by vasoactive peptidyl hormones was almost completely diminished by the pretreatment of the OMCT with a maximal dose of ATP (10^{-4} M) (Table 2). Similar results were obtained under the conditions whereby the extracellular medium contained no calcium. As shown in Fig. 9, these results using ionomycin suggest that the ATP-activated calcium pool was overlapped with intracellular calcium pools activated by vasoactive peptidyl hormones and that ATP prominently caused the depletion of these overlapped intracellular calcium pools. This phenomenon suggests that the complete depletion of the calcium store activated ATP may play a role in any physiological response to agonist stimulation in OMCT.

The cytoplasmic ATP concentration in most cells is assumed to be over 5 mM, and a significant proportion of the cytoplasmic ATP can be released into the extracellular space due to sudden cell death; thus the concentrations of pericellular ATP could easily reach a high enough concentration to stimulate the P2 receptors. The local concentrations of ATP may depend on the amount of ATP released, the distributed fluid volume in the extracellular space, and the levels of activity of the catabolic enzymes, especially the ectonucleotidases present on adjacent cells. Teleological reasoning suggests that this possibility for increases in the extracellular ATP concentrations may be why the range of distribution of ectonucleotidases is wide (29).

In summary, the present study demonstrates that ATP mediates an increase in \([\text{Ca}^{2+}]\) in renal epithelial cells of the definite nephron segments like the glomerulus, S1, CCT, and OMCT. Rank-order efficacy studies of ATP analogs suggest that ATP exerts its action by activation of a P2Y1 purinoceptor in isolated rat S1 and that there are two kinds of P2 purinoceptor (P2Y1 and P2Y2) in the OMCT. ATP might affect calcium mobilizations activated by the vasoactive peptidyl agonists.

This study was supported in part by grants from the Ministry of Education, Science, Sports, and Culture of Japan (0757317 and 08672623), the Science Research Promotion Fund from Japan Private School Promotion Foundation, the Suzukin Memorial Foundation, the Fugaku Trust for Medicinal Research, and the Japan Society for the Promotion of Science for the Japan-Korea Cooperative Science Promotion Program.


Address for reprint requests: H. Endou, Dept. of Pharmacology and Toxicology, Kyorin Univ. School of Medicine, Mitaka, Tokyo 181, Japan.

Received 21 August 1995; accepted in final form 26 January 1998.

REFERENCES


12. Forsberg, E. J., G. Feuerstein, E. Shoahmi, and H. B. Pollard. Adenosine triphosphate stimulates inositol phospholipid metabolism and prostacyclin formation in adrenal medul-


