Effect of extracellular ATP on contraction, cytosolic calcium activity, membrane voltage and ion currents of rat mesangial cells in primary culture

Hermann Pavenstädt, Joachim Gloy, *Jens Leipziger, *Bettina Klär, †Josef Pfeilschifter, Peter Schollmeyer & *Rainer Greger

Department of Medicine, Division of Nephrology, *Department of Physiology, Albert-Ludwigs-University, Freiburg, Germany and †Biocentrum Basel, Switzerland

- 1 The effects of extracellular ATP on contraction, membrane voltage (V_m), ion currents and intracellular calcium activity $[Ca^{2+}]_i$ were studied in rat mesangial cells (MC) in primary culture.
- 2 Addition of extracellular ATP (10^{-5} and 10^{-4} M) to MC led to a cell contraction which was independent of extracellular calcium.
- 3 Membrane voltage (V_m) and ion currents were measured with the nystatin patch clamp technique. ATP induced a concentration-dependent transient depolarization of V_m (ED_{50} : 2×10^{-6} M). During the transient depolarization ion currents were monitored simultaneously and showed an increase of the inward- and outward current.
- 4 In a buffer with a reduced extracellular chloride concentration (from 145 to 30 mM) ATP induced a depolarization augmented to -4 ± 4 mV.
- 5 ATP- γ -S and 2-methylthio-ATP depolarized V_m to the same extent as ATP, whereas α,β -methylene-ATP (all 10^{-5} M) had no effect on V_m .
- 6 The Ca^{2+} ionophore, A23187, depolarized V_m transiently from -51 ± 2 to -28 ± 4 mV and caused an increase of the inward current.
- 7 The intracellular calcium activity $[Ca^{2+}]_i$ was measured with the fura-2 technique. ATP stimulated a concentration-dependent increase of $[Ca^{2+}]_i$ (ED_{50} : 5×10^{-6} M). The increase of $[Ca^{2+}]_i$ was biphasic with an initial peak followed by a sustained plateau.
- 8 The $[Ca^{2+}]_i$ peak was still present in an extracellular Ca^{2+} -free buffer, whereas the plateau was abolished. Verapamil (10^{-4} M) did not inhibit the $[Ca^{2+}]_i$ increase induced by ATP.
- 9 The data indicate that extracellular ATP contracts MC and is able to increase $[Ca^{2+}]_i$ by the release of Ca^{2+} from intracellular stores and recruitment from the extracellular space. In addition ATP depolarizes V_m of MC by activating a Cl^- conductance. The ATP-induced depolarization is mediated by a P_{2y} receptor.

Keywords: Rat mesangial cell; P_{2y} -purinoceptor; contraction; Cl^- conductance; intracellular Ca^{2+} activity

Introduction

Extracellular adenosine 5'-triphosphate (ATP) has been shown to act as a co-transmitter and is able to modulate the function of a great variety of tissues via specific P_2 receptors (Drury & Szent-Györgyi, 1929; Burnstock, 1976; Gordon, 1986; von Kügelgen & Starke, 1991). In the kidney the release of a purinergic transmitter, probably ATP causes the vasoconstrictor response elicited by periaarterial nerve stimulation (Schwartz & Malik, 1989). Therefore, ATP co-secreted with noradrenaline may also act on cells within the kidney. Indeed it has been recently shown that ATP increases $[Ca^{2+}]_i$ in glomerular epithelial- and endothelial cells (Marsden *et al.*, 1990; Pavenstädt *et al.*, 1992) and that mesangial cells respond to ATP in terms of phosphoinositol turnover via a P_2 receptor (Pfeilschifter, 1990a).

In pathological conditions such as glomerular injury, sources of ATP might be damaged cells (Born & Kratzer, 1984), endothelial cells and platelets, which contain high concentrations of ATP in their dense bodies (Gordon, 1986). Platelets are involved in the initial stage of glomerular injury and intraglomerular platelet activation may promote local proliferation of the mesangium by the release of vasoactive

substances (Cameron, 1984; Klahr *et al.*, 1988). Proliferation of MC plays an important role in the inflammatory response to glomerular injury and is a prominent feature of most types of progressive glomerular diseases (Kashgarian & Sterzel, 1992). Recently it has been demonstrated that ATP stimulates proliferation of mesangial cells confirming that ATP might participate in inflammatory processes occurring in glomerulonephritis (Schulze-Lohoff *et al.*, 1992 and own observations).

Little is known about the ability of ATP to contract MC. Furthermore no data about an ATP-induced $[Ca^{2+}]_i$ response and a possible activation of ion currents by ATP are available. The present study investigates whether ATP is able to contract MC and to modulate $[Ca^{2+}]_i$ and ion currents in MC. In addition, the P_2 receptor involved in the ATP response has been characterized.

Methods

Cell culture

MC were cultured as previously described with few modifications (Pfeilschifter *et al.*, 1986). In brief, rat glomeruli were obtained by the sieve technique and incubated with collagenase IV (1 g l^{-1} , Sigma, Deisenhofen, Germany) for

¹ Author for correspondence at Medizinische Klinik, Nephrologische Abteilung, Hugstetterstr. 55, 7800 Freiburg, Germany.

30 min. Glomeruli cores were then suspended in RPMI 1640 medium with 170 g l⁻¹ foetal calf serum, L-glutamine 2.5 mM, sodium pyruvate 0.1 mM, penicillin 10⁵ u l⁻¹, streptomycin 100 mg l⁻¹, non-essential amino acids 0.2% (all Seromed, Berlin, Germany) and insulin-transferrin-sodium selenite supplement 5 mg l⁻¹ (Boehringer-Mannheim, Germany). Fifty glomeruli cm⁻² were plated onto collagen-coated glass cover slips (Greiner, Nürtingen, Germany) and incubated at 37°C in a moist atmosphere containing 5% CO₂. MC outgrowth were characterized morphologically by phase-contrast microscopy, with positive staining for smooth muscle actin, desmin and vimentin. Cytokeratin and factor VIII did not stain, thus excluding glomerular epithelial- or endothelial cells. Furthermore MC contracted in response to angiotensin II. Therefore the cells studied showed the morphological, immunological and physiological properties as described by others for MC (Kreisberg & Karnovsky, 1983). All experiments were done between the 14th and the 30th day with MC in primary culture.

Contraction studies

For contraction studies, MC were kept at 37°C and perfused with a Ringer-like solution to which 5 mM D-glucose was added. MC were continuously observed under an inverted phase-contrast microscope (axiovert 10, Zeiss, Stuttgart, Germany). After addition of ATP, cell contraction was recorded by timed photographs.

Nystatin patch clamp experiments

The patch clamp methods used in these experiments have been described in detail in a previous paper by Greger & Kunzelmann (1991). MC were mounted in a bath chamber on the stage of an inverted microscope, kept at 37°C and perfused with a solution containing (in mM): NaCl 145, K₂HPO₄ 1.6, KH₂PO₄ 0.4, CaCl₂ 1.3, MgCl₂ 1, D-glucose 5, pH: 7.4. The patch pipettes were filled with a solution (in mM) containing K-gluconate 115, KCl 30, Na₂HPO₄ 1.6, NaH₂PO₄ 0.4, CaCl₂ 0.73, MgCl₂ 1.03, EGTA 1, D-glucose 5, pH 7.2, Ca²⁺ activity 10⁻⁷ M to which 10–100 mg l⁻¹ nystatin was added. The patch pipettes had an input resistance of 5 MΩ. A flowing (10 µl h⁻¹) KCl (2 M) electrode was used as a reference. V_m was recorded continuously by a pen recorder (Gould, Seligenstadt, Germany). After achieving a GΩ seal, nystatin slowly permeabilized the cell-attached membrane by inducing a nonselective cation conductance (Marty & Finkelstein, 1975). In 20 experiments the cell attached membrane was only partially permeabilized (cell-attached nystatin = CAN experiments). In these experiments the input current was 0.33 ± 0.03 nS. In 101 experiments in the slow whole cell configuration (with a nystatin concentration > 50 mg l⁻¹) the cell membrane was permeabilized to a greater extent (slow whole cell = SWC configuration), the input current was 8.5 ± 0.9 nS. The V_m value was not different in all series. All experiments were done after a control period of about 5 min. The voltage (V_m) and the current (C) were followed before, during and after addition of the agonists. Currents were measured in the voltage clamp mode of the amplifier. Voltage steps of ± 40 mV (duration: 0.1 s) were applied to estimate cellular ion currents. In ion replacement experiments NaCl was replaced iso-osmotically by the substitution ion. To vary the free Ca²⁺ activity, the solutions were prepared according to established techniques with EGTA and NTA as Ca²⁺ buffers. The Ca²⁺ activity was calculated from a standard equation (Gögelein *et al.*, 1987) and was determined with a Ca²⁺ selective electrode (Radiometer, Copenhagen, Denmark).

[Ca²⁺]_i measurements

Measurement of [Ca²⁺]_i was performed with a set-up described recently by Nitschke *et al.* (1992). In brief, fura-2

fluorescence (excitation wavelengths: 340, 360, 380 nm, emission wavelength: 510 nm) was measured under an inverted fluorescence microscope (bath temperature: 37°C, bath solution changes within 1 s). The set up was based on a high speed filter wheel and a single photon tube (Hamamatsu H 3460-04, Hersching, Germany) allowing a time resolution of up to 200 Hz. MC were incubated with fura-2 (5 × 10⁻⁶ M) for 30 min at 37°C. Experiments were performed with a window including on average 10 cells. The measured noise and autofluorescence of the cells did not exceed 1% of the fura-2 fluorescence and was therefore not corrected. The calibration of the fura-2 fluorescence signal was attempted at the end of each experiment using the Ca²⁺ ionophore ionomycin (1 µM) and low and high Ca²⁺ buffers. [Ca²⁺]_i was calculated from the fluorescence ratio 340/380 nm according to the equation described by Grynkiewicz *et al.* (1985).

The following agents were used: adenosine 5'-triphosphate sodium salt (ATP), α,β-methylene-ATP, A23187, ionomycin, verapamil (Sigma Deisenhofen, Germany), ATP-γ-S tetrasodium salt (Boehringer-Mannheim, Mannheim, Germany), 2-methylthio-ATP tetrasodium salt (Biotrend, Köln, Germany).

Statistics

The data are given as mean values ± s.e.mean (*n*), where *n* refers to the number of experiments. Paired *t* test was used to compare mean values within one experimental series. A *P* value of <0.05 was accepted as indicating statistical significance.

Results

MC contraction in response to ATP

MC contracted within 30 s in response to ATP. In four different preparations for each ATP concentration, 10⁻⁵ M ATP contracted 20–40% and 10⁻⁴ M ATP 70–100% of approximately 40 observed MC in each experiment (Figure 1). The contraction took as long as 10 min to reach a maximum and was independent of extracellular Ca²⁺.

Effect of ATP on V_m and ion currents of MC

In 101 experiments in the SWC configuration, a stable V_m of -49 ± 1 mV and in 20 CAN experiments a stable V_m of -48 ± 2 mV was obtained. Figure 2 shows the histogram of the frequency distribution of the V_m measurements in MC. Because the V_m values were not different in the SWC- or CAN configuration, the data from both series were pooled. ATP (10⁻⁵ M) led to depolarization of V_m from -47 ± 1 mV to -30 ± 2 mV (*n* = 65). The depolarization was transient, i.e. resting V_m was reached again after 1–2 min, although ATP was still present. Figure 3a shows an original recording and the concentration-response curve of the effect of ATP on V_m (Figure 3b). Like ATP, angiotensin II depolarized V_m of MC with an EC₅₀ value of 10⁻⁹ M (*n* = 49, data not shown).

Effect of a reduced extracellular chloride concentration on the depolarization induced by ATP

The presence of a reduced extracellular Cl⁻ concentration (from 145 to 30 mM) did not change V_m of MC significantly (*n* = 9). Under these experimental conditions ATP (10⁻⁵ M) induced a significantly augmented depolarization of V_m to -4 ± 4 mV (*n* = 9). Figure 4a shows an original recording of an experiment of the effect of ATP (10⁻⁵ M) on V_m in the absence and presence of a reduced extracellular Cl⁻ concentration. Figure 4b summarizes the data of the paired experiments.

Figure 1 Phase-contrast microscopy of a glomerulus and rat mesangial cells in primary culture. (a) Cells in resting state; (b) cells 1 min after addition of ATP (10^{-4} M). Calibration bar, 5 μ m.

Figure 2 Frequency distribution of the membrane voltage of MC measured with the nystatin patch clamp technique. $\bar{x} = -49 \pm 1$ mV. $n = 121$.

Figure 3 Effect of ATP on the membrane voltage (V_m) of MC. (a) Original recording of an experiment with ATP (10^{-5} and 10^{-4} M). Initially the voltage hyperpolarizes slowly to a steady state. This is caused by the incorporation of nystatin into the membrane patch. (b) Concentration-response curve for the depolarizing effect of ATP. n shown in parentheses.

Figure 4 Effect of a reduction of the extracellular Cl^- concentration (from 145 to 30 mM) on V_m of MC in the absence and presence of ATP (10^{-5} M). (a) Original recording. (b) Summary of the experiments. Data points were collected at the peak of the depolarization induced by ATP. ATP depolarized significantly more in the presence of a low extracellular Cl^- concentration. $n = 9$.

Effect of ATP on ion currents in MC

In SWC experiments, ATP (10^{-5} M) induced a significant increase of the inward current by $18 \pm 3\%$ during the depolarization (cf. Figure 3a, peak). Thereafter, during the hyperpolarization phase (cf. Figure 3a, repolarization in the presence of ATP) the inward current collapsed. During this phase the outward current increased gradually to a maximum increment of $9 \pm 2\%$ (data not shown).

Characterization of the P_2 receptor

The depolarizing response of V_m to several ATP analogues was used to characterize the P_2 receptor of MC. ATP- γ -S and 2-methylthio-ATP (both 10^{-5} M) depolarized V_m to the same extent as ATP, whereas α,β -methylene-ATP (10^{-5} M) had no effect on V_m (all $n = 7$). In addition preincubation of MC with α,β -methylene-ATP (10^{-5} M, $n = 5$) did not inhibit the V_m -response to ATP. Figure 5 summarizes the data.

The V_m response to ATP is independent of extracellular Ca^{2+}

To examine whether a Ca^{2+} influx from the bath was necessary for the V_m response to ATP, ATP (10^{-5} M) was added to MC in the presence and absence of extracellular Ca^{2+} . Figure 6 summarizes the data: In the absence of extracellular Ca^{2+} ATP depolarized V_m to the same extent (by 25 ± 4 mV) as in the presence of extracellular Ca^{2+} (by 26 ± 4 mV, $n = 10$).

The Ca^{2+} ionophore A23187 stimulates a Cl^- conductance in MC

A23187 (10^{-6} M) depolarized V_m of MC (from -51 ± 2 to -29 ± 4 mV, $n = 20$). During the depolarization a marked increase of the inward conductance by $46 \pm 15\%$ was found (Figure 7a). In a buffer with a reduced extracellular Cl^-

Figure 5 P_2 receptor characterization of MC. Summary of the V_m response to ATP and the synthetic ATP analogues ATP- γ -S, 2-methylthio-ATP (2-m-ATP), and α,β -methylene-ATP (α,β -ATP), all 10^{-5} M. Note that ATP, ATP- γ -S and 2-methylthio-ATP have a significant depolarizing effect, whilst α,β -methylene-ATP is devoid of any effect. C = control. $n = 7$ –9.

Figure 6 Summary of the effect of ATP (10^{-5} M) on V_m of MC in the presence and absence of extracellular Ca^{2+} (buffered with 1 mmol EGTA). Note that the ATP-induced depolarization occurs both in the absence and presence of extracellular Ca^{2+} . C = control.

Figure 7 (a) Summary of the depolarizing response of V_m (●) and the increase in the inward current (○) of MC in response to A23187. A23187 depolarizes and increases the inward current significantly. (b) Summary of the effect of A23187 on V_m in a bath containing normal (145 mM) or reduced (30 mM) extracellular Cl^- concentration. Note that the depolarization induced by A23187 is augmented in the low Cl^- medium. $n = 6$.

concentration (from 145 to 30 mM) the depolarization induced by A23187 was increased to -1.7 ± 2 mV, $n = 7$. Figure 7b summarizes the experiments of the effect of A23187 on V_m with normal and reduced extracellular Cl^- concentration.

ATP increases $[Ca^{2+}]_i$ in MC

Addition of ATP to single MC loaded with fura-2 resulted in a rapid and transient $[Ca^{2+}]_i$ peak followed by a plateau elevation above baseline values (ED_{50} about 5×10^{-6} M). Figure 8a and b show an original recording and the concentration-dependence of the $[Ca^{2+}]_i$ response to ATP. Like ATP, angiotensin II ($n = 9$), vasopressin ($n = 2$) and platelet derived growth factor ($n = 3$) led to an increase of $[Ca^{2+}]_i$ in MC (data not shown). In the absence of extracellular Ca^{2+} , the $[Ca^{2+}]_i$ peak induced by ATP was still present, whereas the plateau was reduced (Figure 9, $n = 9$). In addition, removal of the extracellular Ca^{2+} during the plateau phase of the ATP response led to a rapid decline of $[Ca^{2+}]_i$ towards basal levels. To examine whether a voltage-dependent Ca^{2+} channel was responsible for the ATP-induced plateau phase, the $[Ca^{2+}]_i$ response to ATP was examined in the presence and absence of verapamil (10^{-5} and 10^{-4} M, $n = 7$). Each experiment was performed in a paired fashion. Figure 10 shows that the $[Ca^{2+}]_i$ peak- and plateau induced by ATP was not reduced in the presence of verapamil.

Figure 8 (a) Typical microfluorescence recording of the ATP-induced $[Ca^{2+}]_i$ increase in MC. The fluorescence ratio with 340/380 nm excitation is shown as a function of time. (b) Concentration-response curve of the effect of ATP on $[Ca^{2+}]_i$.

Figure 10 Verapamil did not inhibit the $[Ca^{2+}]_i$ increase induced by ATP. (a) Original experiment on the effect of ATP (10^{-5} M) on $[Ca^{2+}]_i$ in the absence and presence of verapamil (10^{-4} M). (b) Summary of the experiments (C = control, C* = control with verapamil). Asterisks indicate significant differences between controls and ATP peak and plateau values respectively. $n = 7$.

Figure 9 (a) Example of an experiment showing the effect of ATP on $[Ca^{2+}]_i$ in the presence and nominal absence of extracellular Ca^{2+} . Note that the ATP-induced peak is unaltered in the absence of extracellular Ca^{2+} . However, the sustained phase is missing. (b) Summary of the dependence of the ATP-induced $[Ca^{2+}]_i$ peak- and plateau on extracellular Ca^{2+} (C = control, C* = control without extracellular Ca^{2+}). Asterisks indicate significant differences between controls and ATP peak- and plateau values respectively. $n = 5$.

Discussion

The present study demonstrates that extracellular ATP exerts physiological effects, including contraction, stimulation of ion currents and increasing of $[Ca^{2+}]_i$ in MC. MC are smooth muscle-like cells, which contract in response to several agonists, like angiotensin II, vasopressin (Ausiello *et al.*, 1980), endothelin (Badr *et al.*, 1989), histamine (Sedor & Abboud, 1985) and platelet activating factor (Schlondorff *et al.*, 1984). The contraction induced by ATP was independent of the presence of extracellular Ca^{2+} . This fits well to data by others who demonstrated that the vasoactive agonist, angiotensin II, contracted MC independent of extracellular Ca^{2+} (Okuda *et al.*, 1986; Takeda *et al.*, 1988), whereas other groups showed a dependence on extracellular calcium of arginine vasopressin-induced contraction of MC (Venkatachalam & Kreisberg, 1985). The physiological consequences of MC contraction induced by ATP or other vasoactive substances still remains a matter of debate. On one hand, contraction of MC is considered to decrease the capillary loop diameter leading to a decrease of glomerular filtration rate, whilst on the other hand, biomechanical studies propose a more static role of mesangial cells (for review see Mene *et al.*, 1989; Kriz *et al.*, 1990).

The effect of ATP on V_m and ion currents was examined with a modified nystatin patch clamp technique, allowing for the simultaneous recording of V_m and ion currents (Greger & Kunzelmann, 1991). The V_m value of -49 mV in MC measured with this technique is in agreement with V_m values measured by others with conventional glass microelectrodes or by fluorescence techniques (Okuda *et al.*, 1986; Kremer *et al.*, 1989). ATP led to a transient depolarization of V_m to -30 mV, which is probably close to the Nernst equilibrium potential for Cl^- . When extracellular Cl^- concentration was

reduced, the depolarization was increased and Vm depolarized nearly to the new Nernst equilibrium potential for Cl^- . In addition, an increase of the inward current was found during the depolarization. This indicates that in MC the ATP induced depolarization is due to an activation of a Cl^- conductance. An activation of a Cl^- conductance by ATP was shown in epithelial cells (Kunzelmann *et al.*, 1992). In smooth muscle cells and other excitable cells ATP is known to activate cation channels (Benham & Tsien, 1987; Bean, 1992). A Cl^- channel activation in MC by angiotensin II and vasopressin has been reported previously (Okuda *et al.*, 1986; Kremer *et al.*, 1989).

The effect of extracellular ATP is mediated by P_2 -purinoceptors, which are subdivided in P_{2x} and P_{2y} subtypes (Burnstock & Kennedy, 1985; Kenney, 1990). Since specific antagonists of the P_2 receptor subtypes are not available, the rank order of potency of different synthetic analogues of ATP was used to characterize the receptor. The rank order of potency of the agonists inducing a depolarization was $\text{ATP} = \text{ATP-}\gamma\text{-S} = 2\text{-methylthio-ATP} > \alpha,\beta\text{-methylene-ATP} = 0$. Therefore, the receptor involved in the response to ATP showed similarities to the P_{2y} receptor (rank order of potency: $2\text{-methylthio-ATP} > \text{ATP} > \alpha,\beta\text{-methylene-ATP}$). In addition, $\alpha,\beta\text{-methylene-ATP}$, which was shown to desensitize the P_{2x} receptor in other tissues was not able to desensitize the P_2 receptor in MC (for review see Kennedy, 1990). A very similar P_2 receptor was recently described in long-term cultured MC (Pfeilschifter, 1990b). In contrast to our findings others have shown that ATP led to a proliferation of MC via a P_2 receptor different from the P_2 receptor observed in this study ($\text{ATP-}\gamma\text{-S} > \text{ATP} > \alpha,\beta\text{-methylene-ATP} > 2\text{-methylthio-ATP} = 0$) (Schulze-Lohoff *et al.*, 1992). One explanation for these differences might be the fact that long-term effects of ATP on MC proliferation were examined in the latter study, whereas short-term effects of ATP on MC were studied in the present paper. Other explanations might be the existence of various P_2 receptors for different cellular functions in MC or a possible variability of the P_2 receptor in different subclones of long term cultured MC.

The ATP-induced activation of a Cl^- conductance shown here, was mimicked by the Ca^{2+} ionophore, A23187. Recently it has been shown that not only an increase of $[\text{Ca}^{2+}]_i$ but also the activation of protein kinase C by phorbol ester induced a Cl^- conductance in MC (Kremer *et al.*, 1992). Therefore, ATP may not only stimulate a Cl^- conductance in MC via an increase of $[\text{Ca}^{2+}]_i$ but also via an activation of protein kinase C. In fact, we also observed a

marked depolarization of Vm upon stimulation of protein kinase C in MC with phorbol ester (own unpublished observations). In contrast to the ATP-induced depolarization, the phorbol ester-induced depolarization was not transient and lasted for minutes. Since the kinetics of the activation of the Cl^- conductance by ATP and A23187 were very similar, the ATP-induced increase of $[\text{Ca}^{2+}]_i$ may play the dominant role in activating a Cl^- conductance in MC. The effect of ATP on Vm was independent of the presence of extracellular Ca^{2+} , indicating that an intracellular $[\text{Ca}^{2+}]_i$ increase is mostly responsible for the depolarization induced by ATP. This observation fits well with studies revealing the effect of angiotensin II on Vm of MC (Okuda *et al.*, 1986).

To investigate further the cellular mechanisms of the ATP-induced Cl^- conductance $[\text{Ca}^{2+}]_i$ measurements were performed in MC. ATP produced a biphasic $[\text{Ca}^{2+}]_i$ increase with a transient peak and a sustained plateau. The plateau, but not the peak was dependent on extracellular Ca^{2+} . This indicates that ATP is capable of releasing $[\text{Ca}^{2+}]_i$ from intracellular stores, but also stimulates a calcium influx from the extracellular space. The intracellular component of the $[\text{Ca}^{2+}]_i$ increase is a possible result of an ATP-induced accumulation of inositol trisphosphate in MC which was recently reported (Pfeilschifter, 1990a). To determine whether the Ca^{2+} influx induced by ATP was due to an activation of the L-type voltage-dependent Ca^{2+} channel, experiments with verapamil were performed. The ATP-induced Ca^{2+} influx by ATP was not inhibited by verapamil. This indicates that not an L-type voltage-dependent Ca^{2+} channel, but a receptor-operated Ca^{2+} channel might be responsible for the Ca^{2+} influx induced by ATP. A Ca^{2+} influx through receptor-operated calcium channels in MC was recently postulated in response to endothelin and platelet-derived growth factor (Mene *et al.*, 1987; Simonson & Dunn, 1991), whereas the angiotensin II-induced Ca^{2+} influx in MC might be in part due to an activation of a voltage-dependent calcium channel (Takeda *et al.*, 1988).

In summary, ATP regulates important physiological effects such as contraction, activation of ion currents and stimulation of the Ca^{2+} signalling system in MC. Since endothelial cells, platelets and damaged cells are a source of ATP, ATP may be released during glomerular injury and participate in the initial pathogenic insult of MC.

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