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European Journal of Pharmacology 360 (1998) 239–247

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Effects of UTP on membrane current and potential in rat aortic myocytes

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Received 27 April 1998; revised 7 September 1998; accepted 11 September 1998

Abstract

The electrophysiological effects of UTP on freshly isolated rat aortic myocytes were examined using the perforated patch clamp technique. Application of α,β-methylene ATP (αβ-meATP) and UTP, putative P2X and $P2Y_2$ or $P2Y_4$ purinoceptor agonists, induced transient and oscillatory inward currents, respectively. Experiments with Cl^- channel blockers and different external Cl^- concentrations demonstrated that the oscillatory current elicited by UTP is attributable to activation of Cl^- channels. The transient component elicited by αβ-meATP appeared to be responsible for a non-selective cationic current. With internal application of low-molecular-weight heparin, a blocker of inositol 1,4,5-trisphosphate ($InsP_3$), the oscillatory current elicited by UTP was abolished. The oscillatory current was activated in an all-or-none manner by UTP over the concentration range 0.1 and 1 μM and the frequency and amplitude were independent of the UTP concentration. Under current-clamp mode, UTP produced an oscillatory membrane potential. These results show that rat aortic myocytes have at least two types of P2 receptors. Activation of the P2Y receptor by UTP produces $InsP_3$, which releases Ca^{2+} from the store site. The resulting increase in intracellular Ca^{2+} concentration causes the oscillatory Cl^- current and the subsequent membrane potential changes.

Keywords: UTP; Cl^- current; Aorta, rat; Membrane potential oscillation

1. Introduction

The basal tone of vascular smooth muscle is regulated by many factors, such as contracting and relaxant substances coming from nerve endings and the endothelium. It has been reported that stimulation of sympathetic nerves, which releases both adenosine 5′-triphosphate (ATP) and noradrenaline, can produce phasic and tonic contractions in vascular smooth muscle via activation of P2 receptors and α-adrenoceptors on the cell membrane, respectively (Burnstock, 1981; Kennedy et al., 1986; Benham et al., 1987). Pharmacological and electrophysiological studies revealed that (i) ATP activates P2X receptors (P2X) which are coupled to ion channels, (ii) these channels are permeable to Na^+, K^+ and Ca^{2+} and (iii) the activated P2X receptor is rapidly desensitized in the presence of ATP (Benham and Tsien, 1987; Inoue and Brading, 1990). α-Adrenoceptor stimulation results in the formation of inositol trisphosphate ($InsP_3$) through the activation of phospholipase C and thus mobilizes Ca^{2+} from the sarcoplasmic reticulum (Berridge, 1993; Inoue and Kuriyama, 1993; Somlyo and Somlyo, 1994). Non-selective cation currents (I_{cat}) are also activated in the presence of α-adrenoceptor agonist, resulting in depolarization of the membrane potential and opening of the voltage-dependent Ca^{2+} channel (Inoue and Kuriyama, 1993). This activation of signal transduction pathways via stimulation of receptors on the cell membrane increases the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and is responsible for the shortening of smooth muscle cells. It is also well known that an

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0014-2999/98/$ - see front matter
PII: S0014-2999(98)00674-8

increase in $[Ca^{2+}]_i$ directly regulates channel activity such as Ca^{2+}-dependent K^+ and Cl^- channels (Amedee et al., 1990; Sipido et al., 1993; Ganitkevich and Isenberg, 1995; Large and Wang, 1996).

P2 receptors fall into two groups: the intrinsic ion channel, P2X receptors, and the seven transmembrane domain, GTP-binding protein coupled receptors, P2Y receptors (Boarder and Hourani, 1998). Molecular cloning studies indicate that P2X and P2Y receptors can be subclassified in at least seven and five groups, respectively (Boarder and Hourani, 1998). Previous studies suggest that P2X and P2Y receptors are expressed in smooth muscle tissue (Dalziel and Westfall, 1994; Harden et al., 1995). P2X receptor stimulation depolarizes smooth muscle cell via activation of non-selective cation currents (Schneider et al., 1991; Ganitkevich and Isenberg, 1995). P2Y receptor activation is coupled to the mobilization of $[Ca^{2+}]_i$ through the phosphatidylinositol pathway in several tissues (Kalthof et al., 1993; Galietta et al., 1994; Wiley et al., 1995). Although it is known that UTP is a potent agonist for $P2Y_2$ and $P2Y_4$ receptors, the signal transduction mechanism in smooth muscle is not well understood. In particular, the electrophysiological events following the stimulation of P2Y receptors by UTP are not clear in smooth muscle cells. In several vascular smooth muscle cells, not only I_{cat} but also Ca^{2+}-dependent Cl^--currents (I_{Cl-Ca}) are activated by application of certain vasoconstrictors (Pacaud et al., 1989; Large and Wang, 1996). A functional role of I_{Cl-Ca} has been proposed but remains to be established, and the functional significance of the membrane potential change caused by activation of Cl^- current under physiological conditions is not clear. In the present study, ion channel currents activated by P2 receptor stimulation in rat aortic myocytes were examined.

2. Materials and methods

2.1. Cell-isolation

Male Wistar rats weighing 200–300 g were anesthetized by ether or stunned and killed by bleeding. Single aortic cells were dispersed by using collagenase as previously described (Imaizumi et al., 1989). Membrane currents and transmembrane potentials were recorded with standard whole-cell clamp and nystatin-perforated patch methods (Hamil et al., 1981; Horn and Marty, 1989), using a List EPC7 amplifier (List, Germany). Current and voltage signals were stored on video-tape, using a PCM system, and were later captured on an IBM computer by using DT2801A as an analog–digital converter and analyzed with the Cellsoft program.

2.2. Electrophysiological experiments

Experiments were carried out at 35 ± 1°C in a physiological salt solution (PSS) containing (mM) NaCl 137, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2.2, glucose 10, and HEPES 10. The pH was adjusted to 7.4 with 10N NaOH. Ca^{2+}, Mg^{2+}-free Hanks' solution for dispersing cells contained (mM) NaCl 137, KCl 5.4, Na_2HPO_4 0.168, KH_2PO_4 0.44, glucose 5.55 and $NaHCO_3$ 4.17. The pipette solution for recording Cl^- currents contained (mM) Cs-aspartate 110, CsCl 30, $MgCl_2$ 4 and HEPES 10. For the whole-cell clamp or the nystatin-perforated patch method, 0.1 mM EGTA or 50–100 μg/ml nystatin was added to the pipette solution, respectively. Cesium ions were replaced by equimolar potassium ions to record net membrane currents and membrane potentials. Most of the experiments in this study were carried out using Cs^+-rich pipette solution except where mentioned otherwise. The pH in these solutions was adjusted to 7.2 with KOH.

2.3. Measurement of tension development

After connective tissue and adventia were removed carefully, 1-mm lengths of vessel ring were cut out from the aorta. Endothelial cells were removed by rubbing the inner wall of the vessel with a cotton pad. The vessel ring was set in a 4 ml organ bath to measure isometric tension with a force-transducer and perfused with Krebs' solution which was maintained at 37 ± 1°C and gassed with 95% O_2 and 5% CO_2. The composition of Krebs' solution was (mM); NaCl 117, $MgCl_2$ 1.2, $CaCl_2$ 2.2, KCl 4.7, KH_2PO_4 1.2, glucose 14 and $NaHCO_3$ 25.

2.4. Drugs

The following drugs were used: low-molecular-weight heparin (heparin, molecular weight was approximately 6000, Sigma), guanosine triphosphate-3K (GTP, Yamasa), adenosine triphosphate-2Na (ATP, Oriental), uridine triphospate-2Na (UTP, Yamasa), adenosine diphosphate-2Na (ADP, Oriental), caffeine anhydride (Wako, Tokyo), niflumic acid (Sigma), 4,4′-diisothiocyanato-stilbene-2,2′-disulfonic acid disodium (DIDS, Sigma) and nystatin (Sigma). Each drug, except DIDS, niflumic acid and nystatin, was dissolved in distilled water to make 10 mM stock solutions. DIDS, niflumic acid and nystatin were dissolved in dimethylsulfoxide (DMSO, 100 mM as stock). These solvents (distilled water and DMSO) had no effect on membrane currents and potentials. All drug concentrations are expressed as the final concentration in the solutions and the pH of the solution was readjusted after addition of drugs. Nucleotides and other drugs were applied at a constant flow rate of 0.1 ml s^{-1}. A change of

solution could be achieved within 5 s. When heparin was applied intracellularly, the recording of the current was started between 7 and 10 min after rupture of the membrane.

3. Results

3.1. Effects of UTP, αβ-meATP and ATP on membrane currents in aortic myocytes

When the recording pipette was filled with a K-aspartate-rich solution (see Section 2), application of 3 μM UTP elicited oscillatory inward currents at a holding potential of −60 mV (Fig. 1A). Similar oscillatory inward currents were observed after application of UTP when potassium currents were abolished by use of Cs^+-rich pipette solution (Fig. 1B). The oscillatory inward currents during application of 50 μM UTP were observed in more than 80% of cells examined. To obtain the current–voltage (I–V) relationship and the reversal potential of the oscillatory inward currents induced by UTP, a triangular ramp waveform was used as voltage command pulse (22.5 mV/10 ms). Application of the ramp pulse is indicated as vertical dots or lines in original current recordings. The UTP-sensitive component was obtained by subtracting the current in the presence of UTP from that in the absence of UTP. The I–V relationship of the initial inward current in oscillation revealed that the current reversed at −32 mV (the right panel in Fig. 1B, average: -32.8 ± 0.8 mV, $n = 11$). The I–V of the second inward current had a reversal potential (Er) of −33 mV (average: -33.1 ± 1.6 mV; $n = 8$). The Er of the third or later inward current in oscillation was always around −32 mV.

Application of 50 μM αβ-meATP, a selective P2X receptor agonist, induced only one transient inward current but not the following oscillation (Fig. 1C, left). The I–V relationship indicated that the transient current reversed at −4 mV (average: -4.5 ± 1.5 mV; $n = 8$) and exhibited a slight inward rectification (the right panel in Fig. 1C). Exposure to 50 μM ADP also induced only one transient inward current which reversed at -3.4 ± 2.1 mV (not shown, $n = 7$). No oscillatory component was observed in the presence of ADP. When 1 mM Cd^{2+} was added to the external solution, the transient inward current elicited by 50 μM αβ-meATP or ADP was reduced to 61.0 ± 8.7 ($n = 3$) or 55% ($n = 1$) of control, respectively, whereas the oscillatory currents elicited by UTP were not affected by Cd^{2+} (103.0 ± 5.7% of control, $n = 3$). As shown in the left panel of Fig. 1D, application of 50 μM ATP induced a transient inward current followed by inward currents. The I–V relationship of the transient current and the oscillatory inward current is expressed in Fig. 1D, right. The Er of the transient inward current was different from ramp pulse to pulse applied during the component (between −20 and +5 mV). In contrast, the oscillatory inward current evoked by ATP had an Er of -32.3 ± 1.0 mV ($n = 8$).

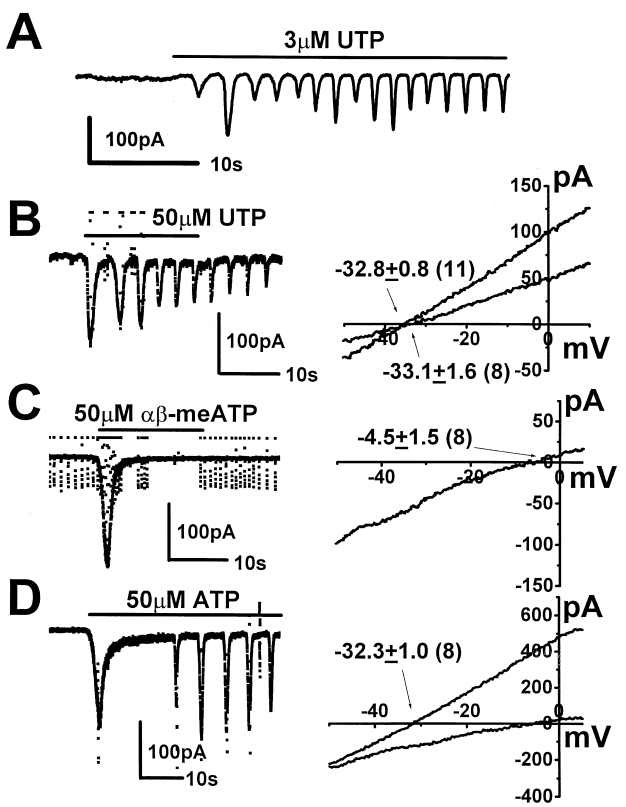

Fig. 1. Effects of UTP, αβ-meATP and ATP on membrane currents in rat aortic myocytes. A: A cell which was internally perfused with K-aspartate-rich solution was held at a holding potential of −60 mV. Application of 3 μM UTP induced a oscillatory inward current. B, C, D: Cs-aspartate-rich pipette solution was used to inhibit K^+ currents. To obtain the reversal potential of the current activated by UTP (B), αβ-meATP (C) and ATP (D), a ramp wave pulse (22.5 mV/10 ms) was applied before and during activation of the current. Vertical dots in B, C and D indicate the application of the ramp pulses. The drug-sensitive current was obtained by subtraction of the current in the absence of each drug from that in the presence and plotted against the test potentials. Each value shown in B, C and D expresses the mean and SEM. Numbers in parentheses denote the number of cells examined.

3.2. Effect of Cl^- channel blockers and change of external Cl^- concentration on the UTP-induced inward current

Under the present experimental conditions, the Cl^- current has a theoretical Er of −36 mV and, therefore, is a relevant candidate for the oscillatory current induced by UTP. The effects of Cl^- channel blockers, niflumic acid and 4,4′-diisothiocyanato-stilbene-2,2′-disulfonic acid disodium (DIDS), on the UTP-induced oscillatory inward

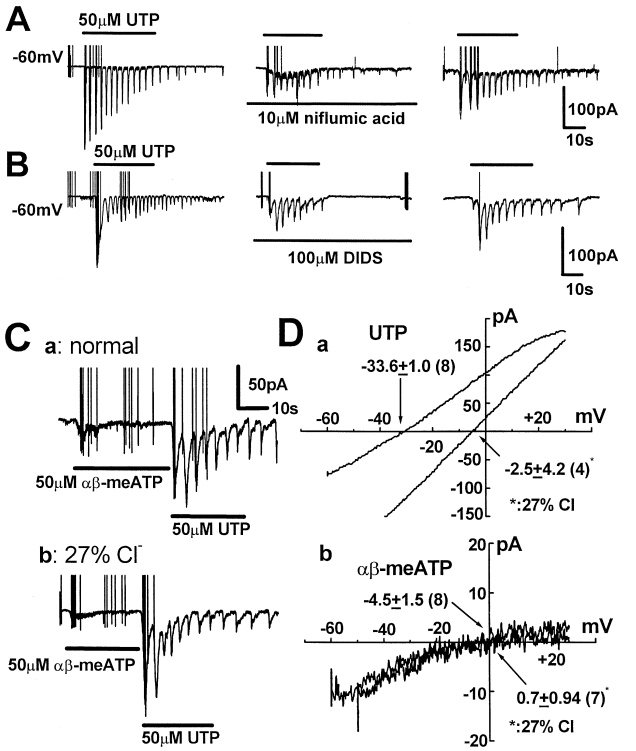

Fig. 2. Effect of Cl^- channel blockers and change of external Cl^- concentration on the UTP-induced inward current. A, B: Cells held at −60 mV were superfused with 50 μM UTP before, during, and after washout of 10 μM niflumic acid (A) and 100 μM 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid disodium (DIDS, B). To block K^+ currents, Cs-aspartate-rich solution was applied to the cell as the pipette solution. During the activation of inward currents, ramp wave pulses were applied several times (vertical lines). C, D: During external perfusion with 150.4 (normal, 'Ca') and 40 mM Cl^- (low Cl^-, 'Cb'), 50 μM αβ-meATP and then UTP were applied. The ramp wave pulses (90 mV/40 ms) were applied to measure the reversal potential of the inward currents activated by each drug. A current–voltage relationship (I–V) was constructed with subtraction of the current in the presence of αβ-meATP or UTP from that in the absence, as illustrated in 'Da or Db', respectively, under normal and low Cl^- conditions. Values in 'D' denote the mean and SEM of the reversal potential. Number of cells examined is shown in parentheses. Note that changing the external Cl^- concentration shifted the reversal potential of I_{UTP} but not $I_{\alpha\beta\text{-meATP}}$.

current were examined (Fig. 2). UTP at the concentration of 50 μM was added before and during the application of 10 μM niflumic acid (A) and 100 μM DIDS (B) and after washout of these drugs. The amplitude of the first UTP-induced oscillatory inward current was markedly reduced in the presence of 10 μM niflumic acid and 100 μM DIDS ($32.5 \pm 5.9\%$, $n = 7$, $P < 0.01$, $31.3 \pm 5.9\%$, $n = 4$, $P < 0.01$, respectively). The inhibitory effects were partially reversed by removal of these blockers ($55 \pm 7.8\%$ of control, $n = 5$, $82 \pm 26\%$, $n = 3$, after washout of niflumic acid and DIDS, respectively). The reversal potential of the UTP-induced current was not affected by niflumic acid (-35.3 ± 0.9 and -35.3 ± 1.1 mV, $n = 4$, in the absence and presence of niflumic acid, respectively).

Fig. 2C, D show the effects of a change in the external Cl^- concentration on Er of the UTP-induced oscillatory inward current. Application of 50 μM αβ-meATP was followed by application of 50 μM UTP. The amplitude of the peak inward current caused by UTP just after application of αβ-meATP was not significantly different from that of the current elicited by UTP alone (-261.4 ± 35.6, $n = 7$, and -218.8 ± 38.6 pA, $n = 12$, respectively, $P > 0.05$). The Er of currents induced by αβ-meATP and UTP ($Er_{\alpha\beta\text{-meATP}}$ and Er_{UTP}) were measured by using a ramp pulse protocol. When the external solution contained 150.4 mM Cl^- (Fig. 2Ca), $Er_{\alpha\beta\text{-meATP}}$ and Er_{UTP} were -4.5 ± 1.5 ($n = 8$) and -33.6 ± 1.0 mV ($n = 8$), respectively. When the external Cl^- concentration was reduced to 27% of the control (40 mM Cl^-) by the replacement of 110.4 mM Cl^- by equimolar aspartate$^-$, $Er_{\alpha\beta\text{-meATP}}$ and Er_{UTP} were -2.5 ± 4.2 ($n = 4$) and 0.7 ± 0.94 mV ($n = 7$) respectively. This shift of Er_{UTP} indicates that a tenfold change in Cl^- concentration resulted in a 54 mV change in Er_{UTP}. In contrast, the same change in Cl^- concentration did not affect $Er_{\alpha\beta\text{-meATP}}$ (-4.5 ± 1.5 and -2.5 ± 4.9 mV in the presence of 150.4 and 40 mM Cl^-, respectively, $n = 4$).

3.3. Effects of heparin and EGTA on the UTP-induced current

It has been reported that the stimulation of P2Y receptors ($P2Y_2$ and $P2Y_4$) by UTP produces inositol-1,4,5-tri-

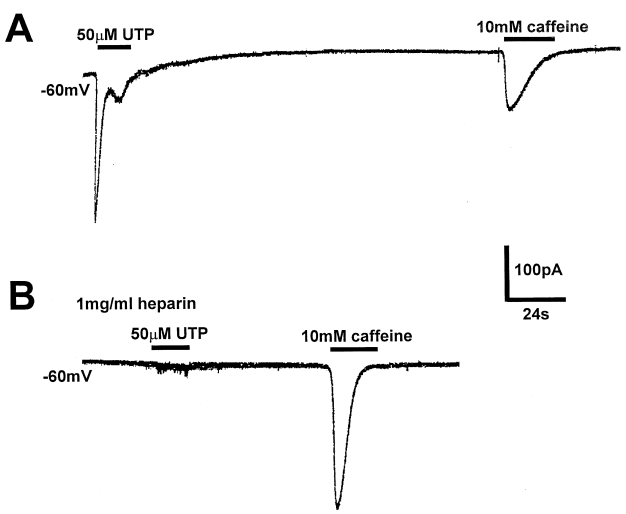

Fig. 3. Effects of low-molecular-weight heparin on the UTP-induced inward current. With (B) and without (A) internal perfusion of 1 mg/ml low-molecular-weight heparin (heparin, MW ~ 6000), 50 μM UTP was applied under whole-cell clamp conditions at a holding potential of −60 mV. Thereafter, cells were treated with 10 mM caffeine. Note that the UTP-induced inward current was not induced in the presence of heparin (B), whereas the inward current was observed under both conditions following application of caffeine.

sphosphate ($InsP_3$) via activation of phospholipase C in several types of cells and releases Ca^{2+} from intracellular storage sites (Kalthof et al., 1993; Galietta et al., 1994; Wiley et al., 1995). To examine whether activation of the UTP-induced inward current is mediated by the formation of $InsP_3$ and subsequent Ca^{2+} release, EGTA or low-molecular-weight heparin (heparin), which chelates Ca^{2+} or inhibits the binding of $InsP_3$ to the receptor on the sarcoplasmic reticulum, respectively, was applied intracellularly from recording pipettes by using the standard whole-cell clamp technique. Under whole-cell clamp conditions, application of 50 μM UTP and then 10 mM caffeine induced the inward current, as shown in Fig. 3A. The reversal potential of the UTP- or caffeine-induced inward current was -34.3 ± 1.1 ($n = 4$) or -33.0 ± 1.2 mV ($n = 3$), respectively. When the pipette solution contained 1 mg/ml heparin, only a small or no response to UTP was observed, whereas the full response to caffeine remained unchanged (Fig. 3B). The averaged amplitude of peak inward currents induced by UTP under the conditions was -584.0 ± 214 pA ($n = 5$) and -13 ± 9.4 pA ($n = 5$) in the absence and presence of 1 mg/ml heparin ($P < 0.05$). The response to UTP was not observed when intracellular Ca^{2+} was buffered by using a pipette solution containing 5 mM EGTA and 0.85 mM Ca^{2+} (pCa 7.5, $n = 3$).

3.4. UTP concentrations and parameters of UTP-induced oscillatory current

Fig. 4 shows the relationships between the concentration of UTP and the UTP-induced oscillatory current at −60 mV in aortic myocytes. Application of 0.1 μM UTP did not affect the membrane current, whereas 1 μM UTP caused an oscillatory inward current (Fig. 4A). Once the oscillatory current occurred, the frequency and peak amplitude were not changed substantially by an increase in UTP concentrations to 10 or 100 μM. The peak amplitude of the UTP-induced oscillatory current in four individual myocytes is plotted against concentration of UTP in Fig. 4B. The oscillatory current was elicited in an all-or-none manner by UTP at concentrations between 0.1 and 1 μM.

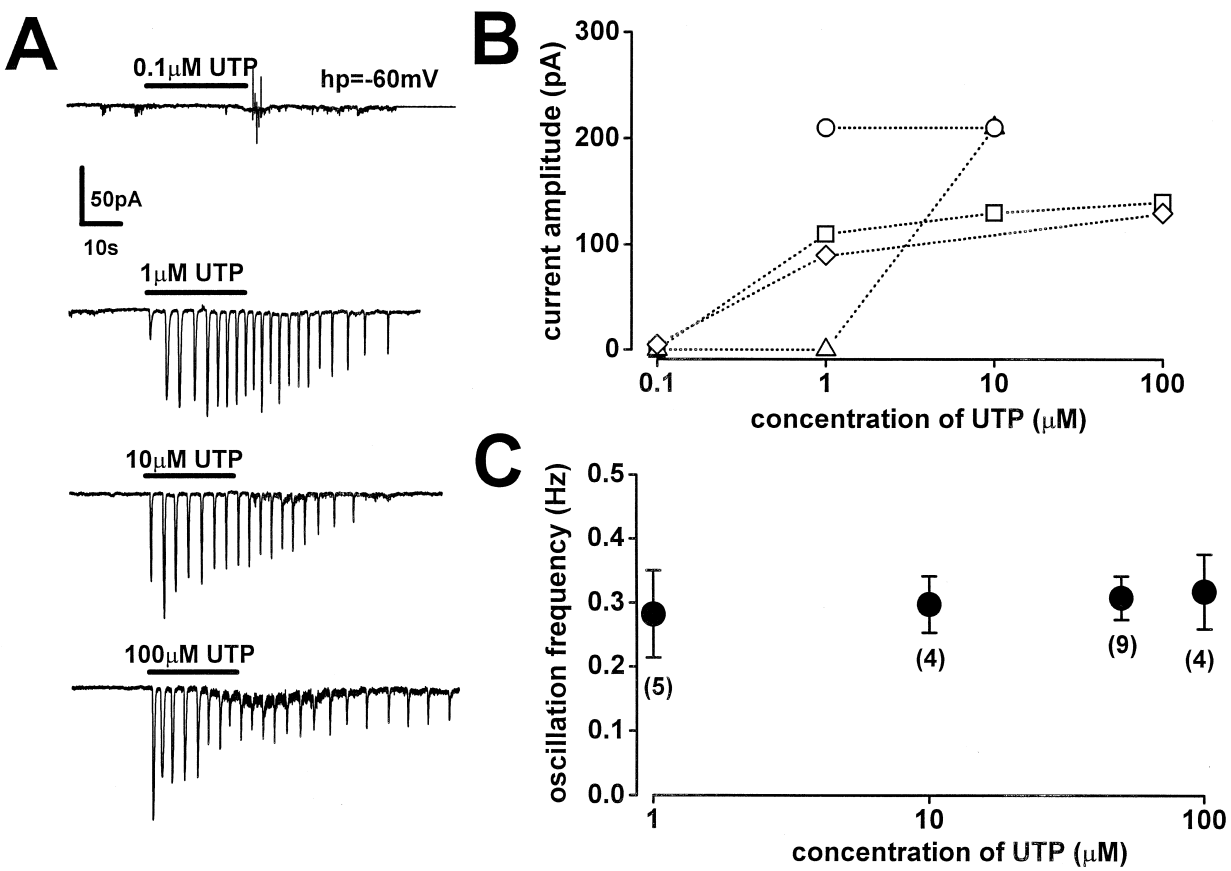

Fig. 4. Relationship between concentration of UTP and the amplitude and frequency of the oscillatory current. Cells were held at a holding potential of −60 mV and 0.1–100 μM UTP was applied. To inhibit K^+ currents, Cs-aspartate-rich solution was used as the pipette solution. Peak amplitude and oscillatory frequency of I_{UTP} were measured and plotted against the concentration of UTP in 'B and C', respectively. Note that the peak amplitude of I_{UTP} was apparently changed in an all-or-none manner by cumulative application of UTP at the concentrations between 0.1 and 100 μM.

The frequency of the oscillatory current obtained at −60 mV was approximately 0.3 Hz at any concentration of UTP between 1 and 100 μM (0.28 ± 0.07, 0.30 ± 0.04, 0.31 ± 0.03 and 0.32 ± 0.06 Hz at 1, 10, 50 and 100 μM UTP, respectively, $n = 4$–9, $P > 0.05$) (Fig. 4C). Moreover, the oscillation frequency of the inward current was independent of the holding potential (frequency of the oscillatory current elicited by 50 μM UTP at −60, −50 and −10 mV: 0.31 ± 0.03, 0.34 ± 0.05 and 0.32 ± 0.06 Hz ($n = 6$–9, $P > 0.05$). When K^+-rich solution was used as the pipette solution, the frequency of the 50 μM UTP-induced oscillation at −50 mV was 0.29 ± 0.06 Hz ($n = 6$).

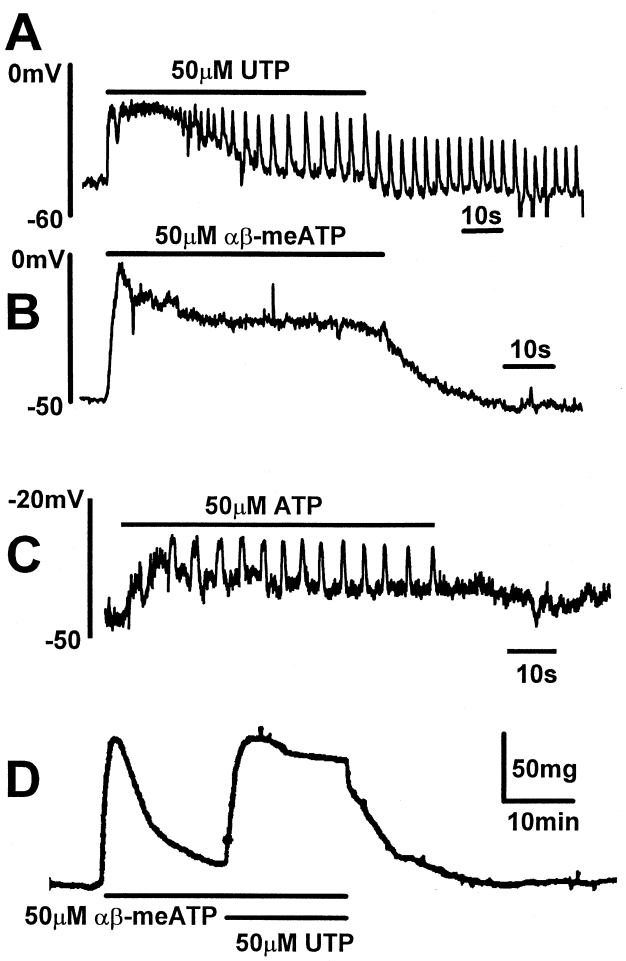

Fig. 5. Oscillatory membrane potential change and tonic contraction caused by UTP. A, B and C: To obtain membrane potential signals, cells were loaded with K-aspartate-rich solution with the nystatin perforated patch clamp technique. Under current-clamp conditions, 50 μM UTP ('A'), αβ-meATP ('B') or ATP ('C') was applied to a rat aortic myocyte. The resting membrane potential of these cells was −48 (A), −49 (B) or −46 mV (C), respectively. D: A tension development produced by αβ-meATP and UTP in rat aortic rings. Endothelial cells were removed by rubbing the inner wall of vessel with cotton pad. Application of 50 μM αβ-meATP and then 50 μM UTP produced the transient and tonic contractions, respectively.

3.5. Oscillation of membrane potential and contraction induced by UTP

Fig. 5A shows the effects of 50 μM UTP on the membrane potential in an aortic myocyte under current-clamp conditions. K^+-rich pipette solution was used. The average resting membrane potential of rat aortic myocytes was −44.2 ± 2.1 mV ($n = 13$). Application of UTP caused a phasic depolarization and subsequent oscillation of the membrane potential. The amplitude of the initial phasic depolarization induced by 50 μM UTP was 23.0 ± 3.4 mV ($n = 8$). The amplitude and the frequency of the membrane potential oscillation were 14.6 ± 3.3 mV and 0.28 ± 0.08 Hz ($n = 5$), respectively. It is notable that the frequency of the membrane potential oscillation was very close to that of the membrane current oscillation under voltage clamp conditions. In the presence of 50 μM Cd^{2+}, similar depolarization and oscillatory membrane potential changes were elicited by addition of 50 μM UTP ($n = 6$). The amplitude of the initial phasic depolarization, the oscillation amplitude and the frequency were not significantly affected by the presence of Cd^{2+}: 23.5 ± 3.1 mV ($n = 6$, $P > 0.05$), 12.5 ± 2.5 mV ($n = 4$, $P > 0.05$) and 0.29 ± 0.09 Hz ($n = 4$, $P > 0.05$). In contrast, a large depolarization (Fig. 5B) (43.5 ± 2.2 mV, $n = 8$) but no oscillation was produced by application of 50 μM αβ-meATP. As shown in Fig. 5C, application of 50 μM ATP elicited a transient depolarization (25.6 ± 4.0 mV, $n = 5$) and oscillation of the membrane potential (amplitude: 10.3 ± 1.2 mV; frequency: 0.22 ± 0.02 Hz, $n = 4$) was recorded.

Fig. 5D shows the effects of 50 μM αβ-meATP and UTP on an aortic ring preparation from which endothelial cells were removed. Application of αβ-meATP induced a phasic contraction and subsequent addition of UTP induced a tonic contraction. The peak amplitude of contractions induced by 50 μM αβ-meATP and UTP was 35.4 ± 4.9 and 23.1 ± 6.7% ($n = 5$) of that of contractions induced by 100 μM noradrenaline, respectively.

4. Discussion

The present study showed that rat aortic myocytes have at least two types of P2 receptor, P2X and P2Y, whose stimulation causes activation of non-selective cation currents (I_{cat}) and Ca^{2+}-dependent Cl^- currents (I_{Cl-Ca}), respectively. Moreover, an oscillatory change of membrane potential due to activation of I_{Cl-Ca} was observed in 80% of myocytes during application of UTP. This is a

clear demonstration that Cl^- current activation can cause membrane potential changes in smooth muscle cells. In addition, the present electrophysiological findings support that UTP can produce a tonic contraction in smooth muscles via activation of P2Y receptors.

4.1. P2 receptors in rat aortic myocytes

The P2X receptor is part of a ligand-gated ion channel which is permeable to Na^+, K^+ and Ca^{2+} and which is expressed in various tissues including smooth muscle (Benham et al., 1987; Dalziel and Westfall, 1994; Boarder and Hourani, 1998). The characteristics of the P2X channel/receptor complex have been extensively studied: inward rectification, non-selective permeability for cations, strong desensitization and an order of potency of nucleotides for receptor stimulation of ATP = ADP ≫ AMP. It is known that αβ-meATP is a selective agonist of the P2X receptor (Dalziel and Westfall, 1994). Our results show that, in rat aortic myocytes, I_{cat} can be selectively activated by application of αβ-meATP, suggesting that stimulation of the P2X receptor is mainly responsible for the initial transient current which is also activated in response to ATP (rat aorta; the present study, rabbit eat artery; Benham et al., 1987, guinea pig urinary bladder; Inoue and Brading, 1990). It is, therefore, possible that activation of the transient I_{cat} produces an initial transient component of the depolarization and the corresponding contraction induced by αβ-meATP. However, as shown in Fig. 5B, 60% of cells had a sustained component of depolarization between −25 and −30 mV in the presence of αβ-meATP. Since a substantial contraction was present even 10 min after application of αβ-meATP (see Fig. 5D), the depolarization which enabled a proportion of the voltage-dependent Ca^{2+} channels to open might have contributed to the induction of the residual contraction after the transient contraction induced by αβ-meATP.

UTP induced the oscillatory current in 65 out of 80 aortic myocytes examined, whereas application of αβ-meATP did not elicit oscillations, suggesting that P2X receptors are not involved in the response. UTP was equipotent or more potent than ATP in eliciting oscillatory currents, indicating that $P2Y_2$ and/or $P2Y_4$ receptor are responsible for the oscillatory currents (Boarder and Hourani, 1998). The reversal potential of the oscillatory currents was approximately −35 mV, which is close to that of Cl^- currents under the present experimental conditions. These results suggest that the oscillatory currents observed in the presence of UTP (also ATP) are due to activation of Cl^- currents via stimulation of $P2Y_2/P2Y_4$ receptors. In the presence of Cl^- channel blockers (10 μM niflumic acid and 100 μM DIDS), the Cl^- current induced by UTP in the present study was not completely blocked. It has been shown that these blockers have a low potency to inhibit Cl^- currents in rabbit portal vein smooth muscle cells (Hogg et al., 1994a,b). Caffeine also activated the Cl^- current, and buffering internal Ca^{2+} at pCa 7.5 with Ca^{2+}-EGTA prevented the activation of the Cl^- current by UTP, indicating that a rise in intracellular Ca^{2+} concentration is required for Cl^- current activation. A similar I_{Cl-Ca} activated by ATP, UTP, noradrenaline, acetylcholine and neurokinin A has been observed in pig (Droogmans et al., 1991) and rat cultured aorta (von der Weid et al., 1993), rabbit ear artery (Amedee et al., 1990) and guinea pig trachea (Janssen and Sims, 1992), respectively.

4.2. Characteristics of I_{Cl-Ca} in aortic myocytes

It has been shown that the Ca^{2+}-induced Ca^{2+} release mechanism but not the $InsP_3$-induced Ca^{2+} release mechanism is involved in activation of the inward current when ATP is applied to rat portal vein (Pacaud and Loirand, 1995). Ca^{2+} influx through I_{cat} may release Ca^{2+} from the sarcoplasmic reticulum via Ca^{2+}-induced Ca^{2+} release mechanism. Our results for rat aorta are in clear contrast with those for rat portal vein. Intracellular application of low-molecular-weight heparin, which inhibits the binding of $InsP_3$ to its receptor in sarcoplasmic reticulum, abolished I_{Cl-Ca}, suggesting that $InsP_3$-formation and subsequent Ca^{2+} release are required for the response to $P2Y_2/P2Y_4$ receptor stimulation. In DDT_1 MF-2 smooth muscle cells, the activation of P2Y receptors by UTP causes the formation of $InsP_3$ (Sipma et al., 1994, 1995). It is likely that $InsP_3$ is one of the signal transducers involved in Ca^{2+} mobilization by stimulation of $P2Y_2$ and/or $P2Y_4$ receptor in DDT_1 MF-2 and aortic smooth muscle cells. Similar results have been obtained for pig aorta (Kalthof et al., 1993).

The membrane potential and also the intracellular Ca^{2+} concentration ($[Ca^{2+}]$) can oscillate in many types of cells following receptor stimulation with agonists (Iino et al., 1994; Nicholls et al., 1995; Yule and Gallacher, 1988). In the present study, neither the amplitude of the oscillatory current nor its frequency was dependent on the UTP concentration. Similar all-or-none current activation and changes in $[Ca^{2+}]_i$ by agonists have been reported in guinea-pig and rat intestinal smooth muscle cells (Ohta et al., 1994). The dependency of the frequency of $[Ca^{2+}]_i$ or I_{Cl-Ca} oscillations on agonist concentration varies in different cell types. The frequency of $[Ca^{2+}]_i$ oscillations is independent of agonist concentration in parotid and pancreatic acinar cells (Yule and Gallacher, 1988) and endothelial cells (Wiley et al., 1995). The frequency of $[Ca^{2+}]_i$ oscillations in rat tail artery and human artery can be accelerated by increasing the concentration of noradrenaline or histamine, respectively (Iino et al., 1994; Nicholls et al., 1995).

4.3. Functional roles of I_{cat} and I_{Cl-Ca}

Several studies have suggested that activation of I_{Cl-Ca} may play an important role in agonist-induced membrane depolarization in smooth muscle cells (Large and Wang, 1996). It has, however, also been argued that the contribution of I_{Cl-Ca} to the depolarization may be small because of its relatively negative reversal potential (~ -30 mV). Our data clearly demonstrate that activation of I_{Cl-Ca} via $P2Y_2/P2Y_4$ receptor stimulation causes a substantial tonic depolarization and superimposed oscillations of the membrane potential under experimental conditions that included a transmembrane gradient of Cl^- concentrations. P2X receptor stimulation by αβ-meATP activated I_{cat} and induced a large transient depolarization, which may facilitate opening of voltage-dependent Ca^{2+} channels. It is possible that the phasic contraction induced by αβ-meATP in rat aorta is dependent on Ca^{2+}-influx through voltage-dependent Ca^{2+} channels.

Acknowledgements

We thank Dr. W. Giles for supplying the data acquisition (AQ) and analysis (Cellsoft) software and giving us valuable comments. This study was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture to K. Muraki.

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