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Action of angiotensin II, 5-hydroxytryptamine and adenosine triphosphate on ionic currents in single ear artery cells of the rabbit

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1 Angiotensin II, 5-hydroxytryptamine (5-HT) and adenosine triphosphate (ATP) evoked a transient inward current in isolated single ear artery cells of rabbit held at −60 mV by whole cell voltage clamp in physiological saline using a KCl-containing pipette solution. Under these conditions agonist did not activate a calcium-dependent potassium current.

2 Responses to each agonist were transient and desensitized rapidly. Inward current at −60 mV holding potential was not abolished by blockade of voltage-dependent calcium channels or by buffering intracellular calcium with BAPTA, a calcium chelator, or following depletion of intracellular calcium stores with ryanodine.

3 The shape of the current-voltage relationships and the reversal potentials of the current induced by angiotensin II, 5-HT and ATP were similar under a variety of ionic conditions. Agonist-induced current was unaffected by replacing intracellular chloride with citrate ions or by replacing intracellular sodium with caesium or extracellular sodium with barium or calcium. Replacement of extracellular sodium with Tris shifted the reversal potential in all cases by around 30 mV negatively.

4 These data suggest that angiotensin II, 5-HT and ATP activate similar cationic conductances which are relatively non-selective allowing mono- and divalent cations to cross the smooth muscle cell membrane. These channels may allow the influx of calcium under physiological conditions.

Keywords: Cation channel; calcium; angiotensin II; 5-hydroxytryptamine; adenosine triphosphate; rabbit artery cells

Introduction

An increase in intracellular calcium ion concentration is a key signal in the initiation of force production in vascular smooth muscle. Contractile agonists are believed to cause an increase in cytoplasmic calcium either by influx of extracellular calcium though calcium-permeable ion channels (Bolton, 1979) or by release of calcium from intracellular stores (Van Breeman & Saida, 1989). It is likely that these mechanisms contribute to a variable degree in mediating the contraction of vascular smooth muscle to different agonists. The ability of contractile agonists to release intracellular calcium stores has been extensively studied in smooth muscle (Van Breemen & Saida, 1989) and is believed to involve the second messenger inositol 1,4,5-trisphosphate (IP_3) which releases calcium from the endoplasmic (sarcoplasmic) reticulum by binding to a specific receptor linked to a calcium release channel (Ehrlich & Watras, 1988). Contractile agonists also depolarize vascular smooth muscle as a result of an increase in membrane sodium (Droogmans *et al.*, 1977) and/or chloride permeability (Wahlström, 1973). Membrane depolarization causes the influx of calcium through voltage-operated calcium channels (Bolton, 1979). Recent studies suggest that agonists in addition to depolarizing the cell membrane potential may also modulate gating of voltage-operated calcium channels in vascular smooth muscle (Benham & Tsien 1988; Nelson *et al.*, 1988; Inoue *et al.*, 1990).

Calcium entry into vascular smooth muscle cells may also occur as a result of activation of receptor-operated channels (Bolton, 1979). These channels have been less extensively studied in vascular smooth muscle. Much of the evidence for their existence is indirect since no drugs which selectively open or block receptor-operated channels have been defined. Many receptor agonists have been shown to increase the influx of calcium by a route distinct from the voltage-operated calcium channel (Bolton, 1979; Cauvin *et al.*, 1987; Zschauer *et al.*, 1987), and at least in some blood vessels this is an important component of the later tonic phase of contraction induced by contractile agonists (Deth & Van Breemen, 1974; Hester, 1988). Although considerable indirect evidence for such a receptor-operated pathway exists there is less direct evidence from electrophysiological studies for the existence of such channels. Increases in cationic conductance have been reported to occur in response to noradrenaline (Amedee *et al.*, 1990a), adenosine triphosphate (ATP) (Benham *et al.*, 1987), and endothelin (Chen & Wagoner, 1991) and single channels permeable to cations have been shown to be activated by α_1-adrenoceptors (Inoue & Kuriyama, 1993), muscarinic receptors (Inoue & Kuriyama, 1993) and purinoceptors (Benham & Tsien, 1987).

Angiotensin II (AII), 5-hydroxytryptamine (5-HT) and ATP are agonists which cause contraction and depolarization of vascular smooth muscle (Somlyo & Somlyo, 1968; Ishikawa, 1985; Suzuki, 1985; Haeusler & De Peyer, 1989). In this study we have investigated the effects of these agents on ionic currents in vascular smooth muscle cells to determine whether their actions involve the opening of receptor-operated channels.

Methods

Adult New Zealand White rabbits were killed by an overdose of intravenous pentobarbitone and single vascular smooth muscle cells were isolated from rabbit central ear artery as previously described (Benham & Bolton, 1986). Short segments (1-2 mm) of artery were incubated for 1 h in a modified physiological salt solution containing 10 μM calcium, 2 mg ml^{-1} bovine serum albumin, 1 mg ml^{-1} collagenase,

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Table 1 A Composition of intracellular solutions (in mM)

	Na	*K*	*Cs*	*Mg*	*TEA*	*ATP*	*Cl*	*EGTA*	*HEPES*	*Citrate*
K-free Na	126	0	0	3	10	2	139	2	5	0
Low-Na KCl	5	134	0	3	0	2	135	0.05	5	0
Cl-free Citrate	10	0	165	0	10	2	0	2	5	55

pH of all solutions was 7.2 at 21–23°C adjusted with NaOH, KOH or CsOH as appropriate.

B Composition of extracellular solutions in (mM)

	Na	*K*	*Ba*	*Ca*	*Mg*	*Tris*	*Glucose*	*TEA*	*HEPES*	*Cl*
PSS	126	5	0	1.7	1.2	0	14	0	11	139
K-free PSS	126	0	0	1.7	1.2	0	14	10	11	139
Na-deficient Tris	10	0	0	1.5	1.2	126	0	10	0	136
High barium	5	0	110	0	0	0	0	10	11	220
High calcium	5	0	0	110	0	0	0	10	110	220

pH of all solutions was 7.4 at 21–23°C adjusted with NaOH.

0.5 mg ml^{-1} papain and 5 mM dithiothreitol. Cells were dispersed by mild agitation in this low-calcium physiological salt solution. After centrifugation the cells were resuspended in normal physiological salt solution (PSS) containing (in mM): NaCl 130, KCl 6, $CaCl_2$ 1.7, $MgCl_2$ 1.2, glucose 14 and HEPES 10.7 buffered to pH 7.4 with NaOH. The cells were stored on cover slips at 4°C and used within 6 to 8 h. The experiments were performed using the whole-cell configuration of the patch-clamp technique (Hamill *et al.*, 1981) by means of a List EPC-7 patch-clamp amplifier. Patch pipettes had resistances of 3 to 5 MΩ. The composition of the various pipette solutions used in these studies is shown in Table 1. Voltage clamp command pulses were generated by a BBC microcomputer via a CED 1401 interface using software written by J. Denbigh, or by an IBM compatible computer via a TL-1 DMA interface using a commerically available programme (PCLAMP, Axon Instruments). Data were filtered at 5 Hz using a low pass filter (Barr & Stroud) and recorded on FM tape and later analyzed off-line after analogue-to-digital conversion.

Drugs and chemicals

The following were used: (-)-202 791 (isopropyl-4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-nitro-3-pyridinecarboxylate) (a gift from Sandoz AG, Basel, Switzerland), adenosine triphosphate (Mg salt, Sigma, Poole, U.K.), angiotensin II (acetate salt, Sigma, Poole, U.K.), 1,2-*bis*(2-aminophenoxy)ethane-*N*,*N*,*N*',*N*'-tetraacetic acid (BAPTA) (Sigma, Poole, U.K.), bovine serum albumin (essentially fatty acid free) (Sigma, Poole, U.K.), collagenase (Worthington, Reading, U.K.), dithiothreitol (Sigma, Poole, U.K.), 5-hydroxytryptamine (creatine sulphate salt, Sigma, Poole, U.K.), papain (Sigma, Poole, U.K.) and ryanodine (Calbiochem, Nottingham, U.K.). Drugs were applied by superfusion to the microscope chamber. All other chemicals were obtained from Sigma (Poole, U.K.).

Results

Application of AII, 5-HT and ATP in the bathing solution induced an inward current in PSS in cells held at a membrane potential of −60 mV and dialysed with a low-Na KCl pipette solution (Figure 1). Responses to agonists were transient (1–4 s duration) despite the continued presence of drug in the chamber.

As has been previously reported for ATP (Benham *et al.*, 1987) no outward current was seen at −60 mV following application of any agonist ($n = 3$ in each case), and, when present, little effect was seen on the frequency of spontaneous outward currents which are believed to be due to opening of calcium-activated K channels (Benham & Bolton, 1986; Benham *et al.*,

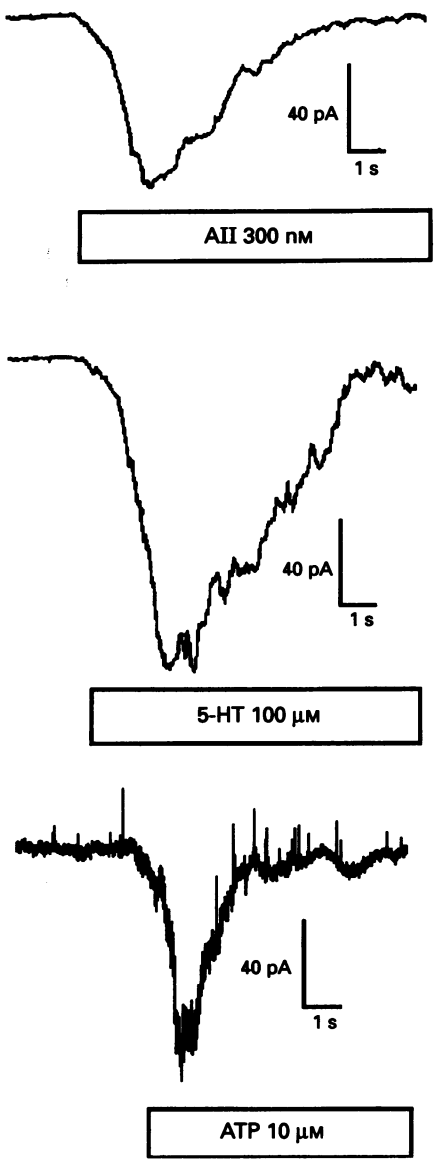

Figure 1 The effect of angiotensin II (AII) 300 nM, 5-hydroxytryptamine (5-HT) 100 μM and adenosine triphosphate (ATP) 10 μM on currents recorded from single rabbit ear arteries cells in physiological saline (PSS). Cells were voltage clamped at −60 mV by whole cell voltage clamp using KCl-containing pipette solution (Table 1). Small spontaneous outward currents (STOCs) are visible in the trace before and after application of ATP. Drugs were present in the bath for the period of time indicated by the bar. Traces are representative of 5–7 similar experiments.

1986). In contrast, noradrenaline (10 μM) and caffeine (20 mM) did produce transient outward currents in the same cells (data not shown) and subsequently suppressed spontaneous outward currents as have been previously reported by others (Benham & Bolton, 1986; Amedee *et al.*, 1990a,b).

In order to allow the inward current to be studied without contamination by potassium currents, further studies were conducted in K-free PSS using a K-free, Na-containing pipette solution (K-free Na) including 10 mM tetraethylammonium chloride in both extracellular and intracellular solutions to reduce potassium conductance (Table 1). Under these conditions AII, 5-HT and ATP produced similar inward currents at a holding potential of −6 mV to those seen previously in cells dialyzed with potassium-containing solution. Responses to all agonists were concentration-dependent (data not shown), but all responses showed homologous desensitization, in that subsequent responses to a similar concentration of the same, but not a different agonist, were reduced, or in the case of higher concentrations, abolished. Consequently, it was only practicable to apply a single concentration of each agonist to an individual cell and concentrations producing near-maximum effects were used for subsequent studies (300 nM AII, 100 μM, 5-HT, 10 μM ATP). Despite the use of maximal or near maximum concentrations of agonist it is unlikely that these are equi-effective concentrations of agonist. Given that

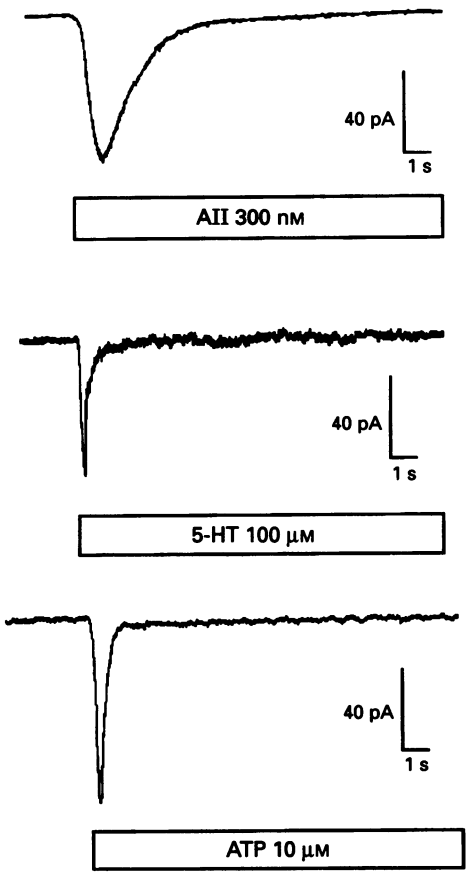

Figure 2 The effect of angiotensin II (AII) 300 nM, 5-hydroxytryptamine (5-HT) 100 μM and adenosine triphosphate (ATP) 10 μM on currents recorded from single rabbit ear arteries cells in K-free physiological saline (K-free PSS) containing 5 μM (-)-202 791, a dihydropyridine calcium antagonist. Cells were voltage clamped at −60 mV by whole cell voltage clamp using K-free Na pipette solution (Table 1) containing an additional 5 mM BAPTA. Drugs were present in the bath for the period of time indicated by the bar. Traces are representative of 3–4 similar experiments.

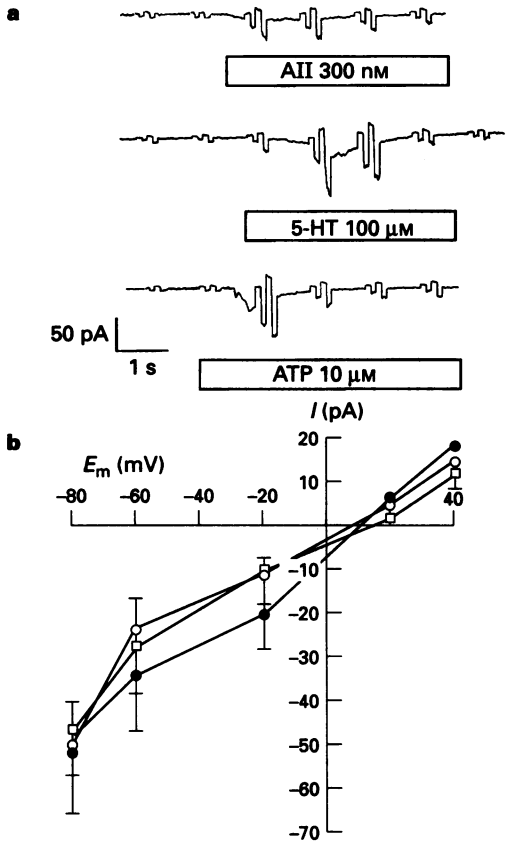

Figure 3 Current-voltage relationship of the currents induced by angiotensin II, 5-hydroxytryptamine and adenosine triphosphate in K-free PSS with a K-free Na pipette solution (Table 1). Cells were voltage-clamped at a holding potential of −20 mV. Every 1 s the holding potential was stepped to +20, −60, +40 and −80 mV for 100 ms at each potential. (a) Shows the effect of angiotensin II (AII) 300 nM, 5-hydroxytryptamine (5-HT) 100 μM and adenosine triphosphate (ATP) 10 μM on currents in the same single cell; (b) shows a plot of the average peak current-voltage relationship induced by the agonists in 5–6 cells: (○) AII; (●) 5-HT; (□) ATP. Points represent medians, vertical bars interquartile ranges.

Table 2 Reversal potentials (E_{rev}) for agonist-induced currents in rabbit ear artery cells under various ionic conditions

Extracellular solution	*Intracellular solution*	E_{rev} (mV) *Angiotensin II*	*5-HT*	*ATP*
K-free PSS	K-free Na	5±2 (6)	6±2 (6)	6±4 (5)
Na-deficient Tris	K-free Na	−24±4 (4)	−30±5 (4)	−24±2 (4)
K-free PSS	Cl-free citrate	7±1 (4)	5±2 (4)	7±1 (4)
High barium	K-free Na	1±2 (4)	2±3 (4)	3±1 (5)
High calcium	K-free Na	−2±4 (4)	−1±1 (4)	5±3 (4)

Reversal potentials (E_{rev}) were estimated by interpolation from the current-voltage relationship of the agonist-induced response as described in Results. Data are means ± s.e.means of (*n*) observations.

the efficacy of coupling between receptor and channel is likely to vary, it is probable that different agonists may induce different degrees of channel opening.

In view of the failure of these agonists to induce a significant outward current in cells dialysed with potassium-containing solution it seemed unlikely that the current induced by these agonists was calcium-activated. However, this possibility was investigated in three ways. A dihydropyridine calcium channel antagonist (-)-202 791 (5 μM) was added to the extracellular solution and the calcium buffering of the intracellular solution was increased by the addition of BAPTA (5 mM) to the K-free Na pipette solution. This concentration of BAPTA was calculated to be sufficient to buffer intracellular concentrations of calcium of up to 50 μM to below 1 nM free ionized calcium concentration on the basis of the affinity constant of BAPTA (Tsien, 1980). Also some cells were treated with ryanodine (100 μM) an agent which depletes intracellular calcium stores in rabbit ear artery cells (Kanmura *et al.*, 1988) by adding it to the extracellular solution and to the pipette solution. None of these procedures abolished responses to AII, 5-HT or ATP, although in some cases the responses appeared to desensitize more rapidly when (-)-202 791 and BAPTA were used (Figure 2).

The current-voltage relationship of the conductance change induced by AII, 5-HT and ATP with a K-free PSS extracellular solution and a K-free Na pipette solution was studied by holding the cells at a membrane potential of −20 mV and stepping to −60, +20, −80, +40 mV for 100 ms. Agonist was applied to the bath while this voltage protocol was repeated every second and the changes in current at the different holding potential were observed. From these data a current-voltage relationship for agonist-induced current was constructed for each stimulant. Current at any potential before application of the agonist was subtracted from current at the potential in its presence to obtain the net current generated by receptor activation at the potential. The voltage-protocol closest to the maximum inward current induced by the agonist as judged from the holding potential of −20 mV was used for this comparison to minimize time-dependent changes in current following application of agonist, but because of the transience of the response to any agonist it was impossible to record the steady-state current-voltage relationship. An example showing individual responses to AII, 5-HT and ATP in the same cell and the current-voltage relationship derived from studies in 5–6 different cells is shown in Figure 3. The reversal potentials (E_{rev}) for the current induced by the three agonists was calculated for each individual cell by interpolation, the mean E_{rev} derived from a number of such similar studies are shown in Table 2. It can be seen that all three agonists induce a current with similar reversal potential (around +5 mV) using PSS extracellularly and a K-free Na intracellular pipette solution.

It has been suggested that contractile agonists may activate a chloride conductance in smooth muscle (Byrne & Large, 1988; Amedee *et al.*, 1990b). Under the conditions described above the E_{rev} for chloride was calculated to be around 0 mV from the Nernst equation so it was possible that a change in chloride conductance was responsible for the currents seen in these cells. To investigate this further, studies were conducted with a caesium citrate-containing pipette (Cl-free citrate; Table 1), thus altering the E_{rev} for chloride from approximately 0 mV to around −66 mV. Under these conditions addition of AII, 5-HT and ATP increased membrane conductance inducing a current which was qualitatively similar to that obtained previously when NaCl was the major intracellular constituent (Figure 4). The calculated E_{rev} derived from these studies in four cells was little different from the E_{rev} obtained with a K-free Na pipette solution (Table 2) suggesting that the current was not due to an increase in chloride conductance.

In a subsequent series of experiments the effect was investigated of replacing extracellular sodium with the large organic cation, Tris, in combination with K-free Na solution in the patch pipette thereby altering the E_{rev} for sodium but leaving the E_{rev} for chloride unaltered. Under these conditions the E_{rev} for the current induced by all three agonists was shifted approximately 30 mV negatively with respect to the E_{rev} in PSS (Figure 5), suggesting that it was carried by cations and that the channel responsible was relatively impermeable to Tris.

To characterize further the cationic conductance induced by the three agonists, studies were conducted using the divalent cations, barium and calcium, to make up the extracellular solutions (Table 1). The results of these studies are shown in Figures 6 and 7. When either barium or calcium was the extracellular cation used in combination with a K-free Na containing pipette solution, the current-voltage relationship was similar to the studies using K-free PSS as the extracellular solution and the E_{rev} for the agonist-induced current was close to zero for all three agonists (Table 2, Figures 6 and 7). These

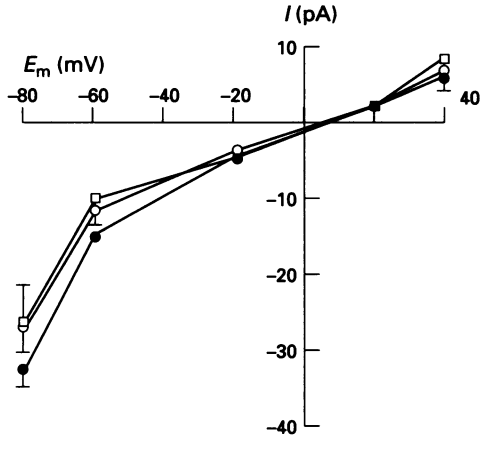

Figure 4 Current-voltage relationship of the currents induced by angiotensin II (AII), 5-hydroxytryptamine (5-HT) and adenosine triphosphate (ATP) in K-free PSS with a caesium citrate pipette solution (Table 1). Cells were voltage-clamped at a holding potential of −20 mV. Every 1 s the holding potential was stepped to +20, −60, +40 and −80 mV for 100 ms at each potential. The graph shows a plot of the average peak current-voltage relationship induced by the agonists in 4 cells: (○) AII; (●) 5-HT; (□) ATP. Points represent medians, vertical bars interquartile ranges.

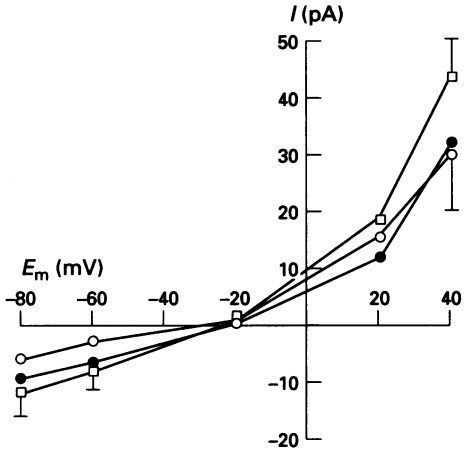

Figure 5 Current-voltage relationship of the currents induced by angiotensin II (AII), 5-hydroxytryptamine (5-HT) and adenosine triphosphate (ATP) in a Na-deficient Tris solution with a K-free Na pipette solution (Table 1). Cells were voltage-clamped at a holding potential of −20 mV. Every 1 s the holding potential was stepped to +20, −60, +40 and −80 mV for 100 ms at each potential. The graph shows a plot of the average peak current-voltage relationship induced by the agonists in 4 cells: (○) AII; (●) 5-HT; (□) ATP. Points represent medians, vertical bars interquartile ranges.

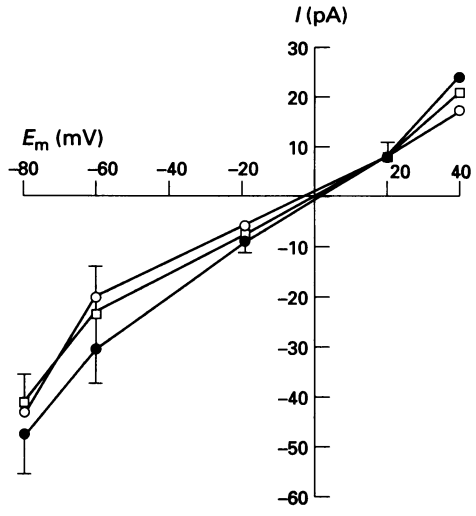

Figure 6 Current-voltage relationship of the currents induced by angiotensin II (AII), 5-hydroxytryptamine (5-HT) and adenosine triphosphate (ATP) in a high calcium solution with a K-free Na pipette solution (Table 1). Cells were voltage-clamped at a holding potential of −20 mV. Every 1 s the holding potential was stepped to +20, −60, +40 and −80 mV for 100 ms at each potential. The graph shows a plot of the average peak current-voltage relationship induced by the agonists in 4 cells: (○) AII; (●) 5-HT; (□) ATP. Points represent medians, vertical bars interquartile ranges.

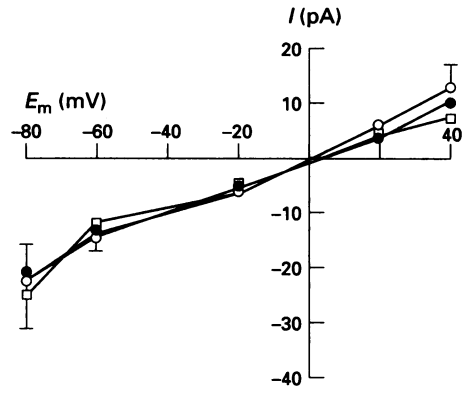

Figure 7 Current-voltage relationship of the currents induced by angiotensin II (AII), 5-hydroxytryptamine (5-HT) and adenosine triphosphate (ATP) in a high barium solution with a K-free Na pipette solution (Table 1). Cells were voltage-clamped at a holding potential of −20 mV. Every 1 s the holding potential was stepped to +20, −60, +40 and −80 mV for 100 ms at each potential. The graph shows a plot of the average peak current-voltage relationship induced by the agonists in 4 cells: (○) AII; (●) 5-HT; (□) ATP. Points represent medians, vertical bars interquartile ranges.

results were interpreted as suggesting that all agonists induced a significant increase in permeability to barium and calcium ions. On the basis of the Goldman-Hodgkin-Katz equation as modified by Lewis (1979) the relative permeabilities of barium to sodium were calculated to be 1.8, 2.2, and 2, and calcium to sodium were 1.5, 1.6 and 2.2 for AII, 5-HT and ATP respectively. Thus AII, 5-HT and ATP induce a relatively non-selective increase in conductance to both mono- and divalent cations.

Discussion

Three contractile agonists AII, 5-HT and ATP which depolarize vascular smooth muscle open channels which have similar properties. Agonist-induced current is carried by cations and the channels responsible appear to be relatively non-selective for cations in that sodium, casesium, barium and calcium are all relatively permeant. Nevertheless the channels appeared to display some weak selectivity for the divalent cations, barium and calcium over the monovalent ion, sodium. The characteristics of the cationic current induced by ATP have previously been described by Benham and colleagues (1987) and our findings in respect to this agonist are similar. No differences were detected between the currents induced by AII and 5-HT or ATP either in terms of ionic permeability or in the shape of the current-voltage relationships under a range of ionic conditions. This suggests either that similar cationic channels are linked to each receptor type or that the three receptor types share the same population of cationic channels.

One hypothesis which could explain the ability of different receptors to open the same receptor-operated channel would be if the receptors mobilized some common second messenger which activated the cationic channel. Our studies appear to preclude intracellular calcium as the common link activating this conductance since buffering with calcium chelator BAPTA did not affect responses to AII, 5HT or ATP. Benham *et al.* (1987) drew similar conclusions regarding the Ca^{2+}-independence of the action of ATP. Thus, the cationic channels involved in these responses seem not to be calcium-activated cation channels, although such channels are present in some vascular smooth muscle (Loirand *et al.*, 1991; Wang *et al.*, 1993) and other cell types (Johns *et al.*,1987; Ehara *et al.*, 1989). Calcium-activated cation channels may be involved in the action of endothelin-1 (Chen & Wagoner, 1991) and noradrenaline in rabbit ear artery (Wang *et al.*, 1993). AII (Griendling *et al.*, 1987), 5-HT (Cohen & Wittenauer, 1987) and ATP (Phaneuf *et al.*, 1987; Tawada *et al.*, 1987) have been reported to elevate inositol 1,4,5 trisphosphate (IP_3) in vascular smooth muscles. Nevertheless only activation of a transient outward current was observed (probably reflecting activation of calcium-activated K channels as a result of intracellular store release) in response to noradrenaline in rabbit ear artery cells when 0.05 mM EGTA was present in the intracellular solution. Our findings suggest that AII, 5-HT and ATP do not cause substantial release of calcium from intracellular stores under the conditions of the study. This and the presence of 2 mM EGTA in the pipette solution may account for the failure of these agonists to increase chloride conductance in these cells or to activate the monovalent ion-selective channel reported by Wang and colleagues (1993), since this, like the potassium conductance, is calcium-dependent. In T-lymphocytes (Kuno & Gardner, 1987) and human epidermoid carcinoma cells (Mozhayeva *et al.*, 1990) IP_3 directly opens a calcium permeable channel. It is possible that IP_3 directly mediates cation channel opening in rabbit ear artery cells. However, if so, then the rise in IP_3 in the cell would be expected to have discharged intracellular calcium stores, raised intracellular calcium and opened calcium-activated K channels, but this was not seen.

The possibility that G proteins might mediate opening of receptor-gated channels in rabbit ear artery is more likely. AII, 5-HT and ATP are all metabotropic receptors known to couple to heterotrimeric G proteins (Birnbaumer *et al.*, 1990). G proteins are known to modulate the activity of a number of ion channels (Clapham, 1994) and acetylcholine has been reported to open a cation channel in guinea-pig ileal smooth muscle (Inoue & Isenberg, 1990a; Komori *et al.*, 1992) through a G protein-dependent mechanism. Intracellular dialysis of rabbit portal vein cells with the non-hydrolysable analogue of GDP, GDP-β-S has been reported to reduce ATP-induced cation currents (Xiong *et al.*, 1991), and Oike and colleagues (1993) reported that a pertussis toxin-sensitive G protein participated in the activation of a cation current by phorbol 12,13-dibutyrate, a protein kinase C activator. Further studies will be necessary to explore the possibility that G proteins also mediate the effects of AII, 5-HT and ATP on channels in vascular smooth muscle cells.

The properties of the channel activated by AII, 5-HT and ATP appear to differ from cation channels found elsewhere. The AII-, 5-HT- and ATP-activated channel is considerably less permeable to divalent compared with monovalent ions than the cation channel activated by NMDA in neural cells (Iino *et al.*, 1990), or the calcium-activated cation channel described in vascular smooth muscle by Loirand and colleagues (1991). The channels activated by AII, 5-HT and ATP show similar permeabilities for monovalent and divalent cations to the channel activated by acetylcholine in guinea-pig ileum, but differs in terms of the effect of membrane potential on conductance (Benham *et al.*, 1985; Inoue & Isenberg, 1990b). The channels activated by AII, 5-HT and ATP also show some differences from the non-selective cation channel activated by noradrenaline which has been described in portal vein (Wang & Large, 1991) and in ear artery cells (Amedee *et al.*, 1990a,b). As pointed out by these workers, the cationic conductance opened by noradrenaline undergoes marked inward rectification over the range −30 to −50 mV, whereas the current-voltage relation for AII, 5-HT and ATP appears almost linear over this range. Furthermore, at least in portal vein, the cation channel activated by noradrenaline is more permeable to barium compared with sodium (approximately 5–12:1) than we describe here (approximately 2:1). Recently, Inoue & Kuriyama (1993) have reported that α_1-adrenoceptor and muscarinic receptor activation opens sodium permeable channels with similar single channel conductance in rabbit portal vein. A similar conductance cation channel activated by ATP was earlier described by Benham & Tsien (1987) in rabbit ear artery. The relationship of these channels to the channels activated by AII and 5-HT remains to be established.

At present the physiological role of the receptor-operated increase in cationic conductance induced by the three agonists is unknown. Under physiological conditions assuming a resting potential of −60 mV, 130 mM Na^+ and 1.8 mM Ca^{2+} outside the cell, and a P_{Ca}/P_{Na} of approximately 2, approximately 90% of the current would be carried by Na^+ ions (though whether this calculation would apply in a mixture of ions is questionable). Nevertheless this prediction is consistent with the ability of agonists such as AII to increase Na^+ permeability in vascular smooth muscle (Aboulafia *et al.*, 1989) and it seems likely that in some tissues cationic channel opening may play an important role in agonist-induced depolarization and may also contribute to calcium entry in some vascular smooth muscle. A recent study (Benham, 1989) using indo-1 as an indicator of cytoplasmic calcium concentration has confirmed that ATP causes significant influx of calcium through a cation channel in rabbit ear artery cells in the presence of physiological levels of extracellular calcium.

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