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Evidence that acetylcholine-mediated hyperpolarization of the rat small mesenteric artery does not involve the K^+ channel opened by cromakalim

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1 Acetylcholine causes a concentration-dependent hyperpolarization of the rat small mesenteric artery (diameter at 100 mmHg, 200–400 μm). In the absence of tone the average potential change was from approximately −60 to −75 mV. In the presence of tone induced by endothelin-1 (20 nM), acetylcholine caused vasorelaxation in association with a marked hyperpolarization; from approximately −32 to −71 mV.

2 A number of compounds known to antagonize the actions of cromakalim were tested for their ability to block responses to acetylcholine. Glibenclamide (0.1–3 μM), phentolamine (10–100 μM) and alinidine (1–30 μM) caused a concentration-dependent depolarization of the rat small mesenteric artery which was not dependent on an intact endothelium. Glibenclamide was approximately 10 times more potent than either phentolamine or alinidine, a similar ratio to their potency as antagonists of cromakalim.

3 In the presence of concentrations of the cromakalim antagonists which functionally inhibited responses to cromakalim, only phentolamine and alinidine had a significant effect on the hyperpolarization and functional responses to acetylcholine. Glibenclamide was without effect at the concentrations used.

4 Experiments on pig coronary artery, where acetylcholine causes vasoconstrictor responses, showed that phentolamine and alinidine have some anti-muscarinic activity which could account for their ability to affect vasorelaxant/hyperpolarization responses to acetylcholine in the rat small mesenteric artery.

5 The results suggest that the acetylcholine-mediated hyperpolarization observed in the rat small mesenteric artery does not involve K^+ channels opened by cromakalim. This finding differs from other studies performed on the rabbit middle cerebral artery which show hyperpolarizing responses to acetylcholine to be glibenclamide-sensitive. It is likely therefore that the hyperpolarization response observed to acetylcholine can be initiated through a number of mechanisms, only one of which utilizes K^+ channels opened by cromakalim.

Keywords: Acetylcholine hyperpolarization; EDHF; rat small mesenteric artery; K^+ channels; cromakalim

Introduction

Acetylcholine-induced vasorelaxant responses have been shown to result from the release of vasoactive intermediates from the endothelium (see Angus & Cocks, 1989). The principle vasoactive substance, endothelium-derived relaxing factor (EDRF), is thought to be nitric oxide (NO) or a derivative since the functional actions of acetylcholine are inhibited by compounds known to capture NO, such as haemoglobin (Angus & Cocks, 1989). However, acetylcholine and other cholinomimetics have been shown to hyperpolarize vascular smooth muscle (Bolton *et al.*, 1984; Chen *et al.*, 1988; Feletou & Vanhoutte, 1988). Again, this action is dependent on an intact endothelium which indicates the involvement of an intermediate. However, in contrast to the vasorelaxant effects of acetylcholine, the electrophysiological effects are not blocked by haemoglobin or methylene blue (Chen *et al.*, 1988; Nishiye *et al.*, 1989). This finding has prompted a number of investigators to propose the release of an endothelium derived hyperpolarizing factor (EDHF; see Taylor & Weston, 1988).

The mechanism behind the hyperpolarization observed with acetylcholine may depend on the origin of the vessel. Some studies have shown that the hyperpolarization response is inhibited by ouabain which suggests the involvement of Na^+/K^+ ATPase (Feletou & Vanhoutte, 1988; Brayden & Wellman, 1989). However, the majority of other studies suggest that an increase in K^+ conductance is responsible (see Chen *et al.*, 1989). While there are many types of K^+ channels, recent studies by Standen *et al.* (1989) showed that the hyperpolarizing actions of acetylcholine and a number of other vasodilators, were sensitive to glibenclamide, a compound which antagonizes ATP-sensitive K^+ channels. This result supports the idea that hyperpolarization is the result of increased K^+ conductance. Cromakalim, pinacidil and a number of other compounds termed K^+ channel openers are also thought to produce membrane hyperpolarization by opening the same glibenclamide-sensitive channel (see McPherson & Angus, 1990). Thus it would appear that a number of endogenous ligands use this particular channel to induce membrane hyperpolarization.

In the rat small mesenteric artery we noted that glibenclamide and two other novel antagonists of the actions of cromakalim, phentolamine and alinidine (McPherson & Angus, 1989), caused direct depolarizing effects on the smooth muscle cell. This observation was not made by Standen *et al.* (1989) in their work on the rabbit middle cerebral artery. Consequently we extended our study on the rat small mesenteric artery to examine the actions of the cromakalim antagonists in greater detail and the interaction between these compounds and the vasorelaxant and hyperpolarizing effect of acetylcholine. We found that there was no correlation between the ability of these compounds to block K^+ channels opened by cromakalim and their ability to influence the response to acetylcholine. Thus it would appear that in the rat small mesenteric artery, in contrast to the rabbit middle cerebral artery, acetylcholine-induced hyperpolarization is not mediated through K^+ channels which can be opened by the exogenous ligand, cromakalim.

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Methods

Isolation of resistance blood vessels

Wistar Kyoto (WKY) rats were killed by CO_2 asphyxia. The mesentery was rapidly removed and placed in ice cold Krebs solution (composition in mM: NaCl 119, KCl 4.7, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, $CaCl_2$ 2.5 and glucose 11) gassed with 5% CO_2 in O_2. Two mm segments were mounted in a small vessel myograph as previously described (Angus *et al.*, 1988). Briefly, two 40 μm wires were threaded through the lumen of the vessel segment. One wire was attached to a stationary support driven by a micrometer while the other was attached to an isometric force transducer which measured force development. Force development was recorded on a dual flat bed recorder (W&W Scientific Instruments, model 320). Vessels were allowed to equilibrate under zero tension for 30 min. By use of the diameter of the vessel, calculated from the distance between the two mounting wires, a passive diameter-tension curve was constructed as previously described (Mulvany & Halpern, 1977). From this curve the effective transmural pressure could be calculated. The vessel was set at a tension equivalent to that generated at 0.9 times the diameter of the vessel at 100 mmHg. Non-linear curve fitting of the passive length-tension curve was achieved with custom written programmes for the IBM PC (NORMALIZE) which uses the Marquart-Levenberg modification of the Gauss-Newton technique (McPherson, 1985). Vessel diameters at an equivalent transmural pressure of 100 mmHg (D_{100}) are given in the text.

Electrophysiology

In experiments where the intracellular resting membrane potential was monitored, the vessel was mounted as just described. A conventional glass electrode (1 mm blanks, World Precision Instruments Inc., New Haven, U.S.A.) filled with 0.5 M KCl (tip resistance approximately 100 MΩ) was used to impale a single smooth muscle cell. The microelectrode was mounted on a Burleigh Inchworm motor controlled by a 6000 series controller (Burleigh, U.S.A.). The microelectrode was advanced by 0.5 μm steps until impalement was achieved.

The bath containing the vessels (7 ml volume) was part of a 25 ml recirculating system which contained a jacketed organ bath where the Krebs solution was warmed and oxygenated. Drugs could also be added at this site. This design allowed cumulative concentration-effect curves to be constructed when assessing the electrophysiological effects of the various drugs. In some experiments the vessel was activated with a submaximal concentration of endothelin-1 ($\approx$20 nM). In these experiments membrane potential and active tension development changes were recorded simultaneously.

In some experiments the effect of acetylcholine was assessed on vessels which had their endothelium removed mechanically. Thus a polyethylene suture (6/0 prolene, Ethicon) was advanced into the lumen of vessel mounted on the myograph wires. The wires were then separated slightly to bring the vessel into contact with the suture. The suture was moved along the lumen and back eight times. The vessel was then relaxed again and rotated through 90 degrees and this procedure repeated. The viability of the vessel after this procedure was assessed by measuring the response to KPSS before and after rubbing. The effectiveness of the procedure in removing the endothelium was assessed functionally by monitoring the response to acetylcholine (10 μM).

Studies of the pig large coronary artery

Pig right coronary artery was dissected from hearts obtained at an abattoir. Ring segments, 4 mm in length, were mounted in a large vessel myograph and placed under 4 g resting force. A single concentration-effect curve was then constructed to acetylcholine, which in this tissue causes a vasoconstrictor response, in the absence or in the presence of either phentolamine (100 μM) or alinidine (30 μM).

Data collection

Tension and membrane potential data were captured by use of the custom written programme DIGISCOPE (G.A. McPherson) for the IBM PC. This programme uses a DASH16 A/D card (Metrabyte, U.S.A.) which collected and displayed the data at 200 Hz. The data were saved on hard disk and reproduced on a Hewlett Packard 7470A plotter.

Statistics and data analysis

Statistical comparisons between two groups were made by Student's t test. Multiple comparisons between independent samples were made by Scheffe's test (see Wallenstein *et al.*, 1980). Results in the text are the mean $\pm$ s.e.mean for the specified number of experiments.

Drugs

The following drugs were used: acetylcholine bromide (Sigma); endothelin-1 (ET-1, Austpep, Australia); phentolamine mesylate (Ciba-Geigy); alinidine bromide (Boehringer-Ingelheim); glibenclamide (Hoechst).

Results

Characteristics of the electrophysiological and functional effects of acetylcholine on the rat small mesenteric artery

The rat small mesenteric artery ($D_{100} = 312 \pm 32$, $n = 7$) had a resting membrane potential of -60 ± 1 mV ($n = 7$, 7 different vessels) in the absence of any vasoactive substance. Acetylcholine (0.01–1 μM) caused a concentration-dependent hyperpolarization of the smooth muscle in the absence of active tone. The membrane potential in the presence of supramaximal concentrations (>0.1 μM) of acetylcholine was -75 ± 2 mV; a hyperpolarization of approximately 15 mV.

Figure 1 shows representative traces of tension and membrane potential, recorded simultaneously, in a mesenteric vessel ($D_{100} = 297$ μm) which had previously been contracted with endothelin-1 (20 nM). Endothelin-1 caused a depolarization to -32 ± 2 mV ($n = 3$) of the vessel in association with a contractile response. Acetylcholine again caused a concentration-dependent hyperpolarization to -71 ± 4 mV in the presence of the highest concentration of acetylcholine used (10 μM); a hyperpolarization of 39 ± 4 mV. The absolute value for membrane potential in the presence of acetylcholine (10 μM) was similar whether in the absence or in the presence of tone induced by endothelin-1 (i.e. -75 versus -71 mV, respectively). The average results show a close correlation between concentrations of acetylcholine required to cause membrane hyperpolarization and relaxation (Figure 2). Since it was experimentally difficult to maintain impalements of cells that were undergoing active tension changes the majority of the studies examining the effects of acetylcholine were performed in vessels which possessed no active tone.

Direct effect of the antagonists of cromakalim on membrane potential and the role of the endothelium

All three cromakalim antagonists studied (glibenclamide 0.1–3 μM, alinidine 1–30 μM and phentolamine 1–100 μM) caused a direct membrane depolarizing effect. The direct membrane depolarizing action of phentolamine is shown in Figure 3. The maximum depolarization produced by glibenclamide (3 μM),

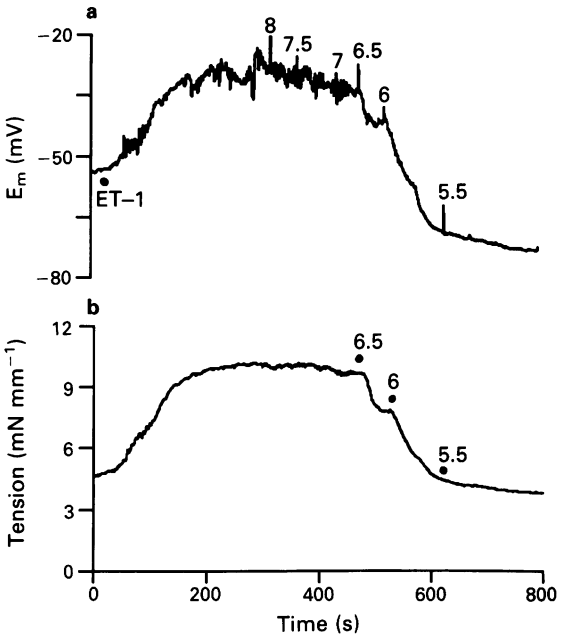

Figure 1 Representative trace obtained in a rat small mesenteric artery in which membrane potential (a) and tension development (b) were recorded simultaneously. Tone was induced with endothelin-1 (ET-1; 20 nM) and the electrical and vasonconstrictor response were followed until they reached a plateau. Thereafter a cumulative concentration-effect curve to acetylcholine was constructed. Concentrations of acetylcholine are marked on the figure as −log (M).

alinidine (30 μM) and phentolamine (100 μM) was 9 ± 1 mV (n = 6), 7 ± 1 mV (n = 7) and 9 ± 2 mV (n = 7) respectively (Figure 4a). The concentration of antagonist required to cause 50% (pD_2 = −log EC_{50}) of the maximum depolarization was calculated graphically (Figure 4b). Glibenclamide (pD_2 = 6.32 ± 0.07) was significantly more potent ($P < 0.05$, Scheffe's test) at causing depolarization by a factor of 5–10 than alinidine (pD_2 = 5.58 ± 0.1) or phentolamine (pD_2 = 5.35 ± 0.19) which were equipotent (Figure 4b).

We observed some dependence of the maximal level of depolarization caused by the antagonists, and the initial resting membrane potential

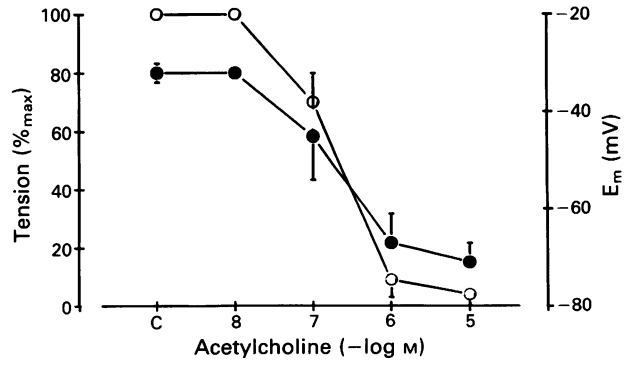

Figure 2 Mean concentration-effect curves for acetylcholine constructed in rat small mesenteric arteries precontracted with endothelin-1 (20 nM). Tension (% E_{max}; left scale, ○) and resting membrane potential (E_m; right scale, ●) were recorded simultaneously. C is the control tension and membrane potential values obtained in the absence of any acetylcholine. Values are the mean for 3 separate determinations; s.e.mean shown by vertical bars.

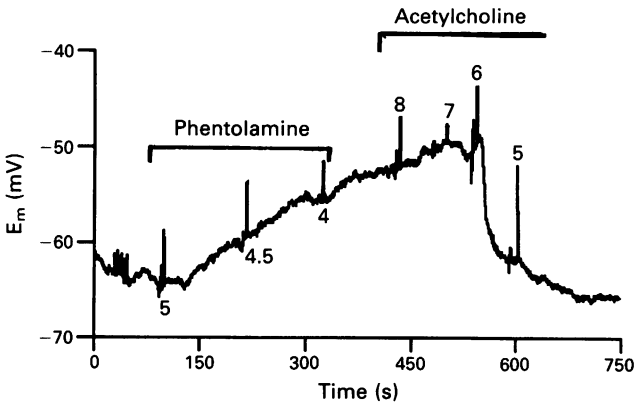

Figure 3 Actual trace showing the effect of increasing concentrations of phentolamine on resting membrane potential (E_m) of a rat small mesenteric artery. At the end of the experiment a cumulative concentration-effect curve was constructed to acetylcholine which hyperpolarized the vessel. Concentrations of drugs are given as −log (M) and indicated by spike marks.

($P < 0.05$, r = 0.67, n = 20). Extrapolation of this regression indicated that the resting membrane potential at which the antagonists had no effect would be approximately −50 mV (see Figure 5a). We also examined whether the degree of depolarization depended on vessel size in this series of experiments and found that there was no correlation ($P > 0.05$, r = 0.09, n = 20) in vessels that ranged in diameter from ≈200–470 μm (Figure 5b).

Vessels were rubbed with the nylon suture to remove the endothelium. In each tissue the maximum response to noradrenaline (10 μM) was assessed before and after the rubbing procedure. The tissue response to noradrenaline after rubbing was 67 ± 1% of the pre-rubbing response indicating some degree of vascular damage. After rubbing, the vessels (D_{100} = 329 ± 15 μM, n = 6) had a resting membrane potential of −51 ± 2 mV (9 impalements from six different vessels) which was significantly different (unpaired t test, $P < 0.05$)

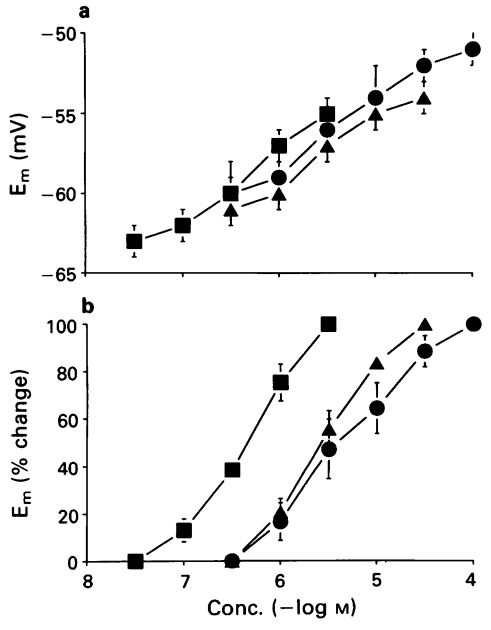

Figure 4 (a) Mean concentration-effect curves constructed in rat small mesenteric arteries assessing the ability of glibenclamide (■), phentolamine (●) and alinidine (▲) to depolarize directly the smooth muscle cell. (b) Standardized mean concentration-effect curves. Membrane potential changes have been expressed as a percentage of the maximum membrane potential change caused by the highest concentration of antagonist used. Results are the mean obtained from 6–7 separate experiments; vertical bars show s.e.mean.

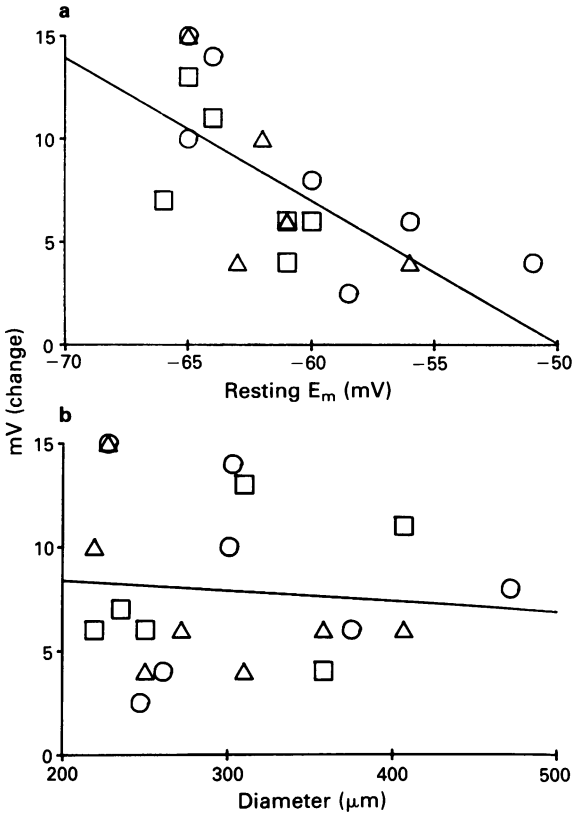

Figure 5 Correlation between the maximum change in resting membrane induced by glibenclamide (□, 3 μM), phentolamine (○, 100 μM) and alinidine (△, 30 μM) and the initial resting membrane potential (a) and also the diameter of the vessel at 100 mmHg transmural pressure (b).

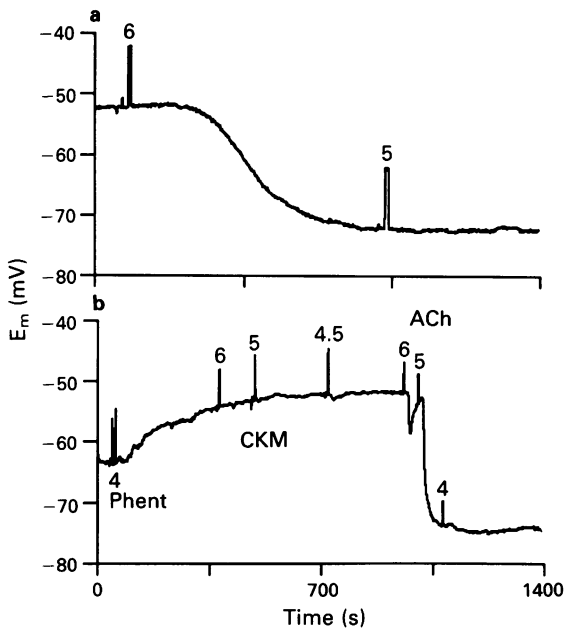

Figure 6 Original records of membrane potential (E_m, mV) in two rat small mesenteric arteries with intact endothelium. (a) Experiment showing the hyperpolarizing effect of cromakalim (−log M) indicated by spike marks. (b) Effect of phentolamine (Phent, 4) on responses to cromakalim (CKM) and acetylcholine (ACh) added cumulatively as indicated. Concentrations are given as −log (M).

from that in unrubbed vessels (−60 ± 1 mV; see earlier). In the absence of endothelium, acetylcholine (10 μM) failed to elicit any hyperpolarization indicating successful removal of the endothelium. However, all cromakalim antagonists still caused significant depolarization (glibenclamide 3 μM, 4.3 ± 0.3 mV; alinidine 30 μM, 3 ± 1 mV and phentolamine 100 μM, 6 ± 1 mV; n = 3–4). None of these values was significantly different from unrubbed vessels although the degree of the depolarization was less than that observed in tissues with an intact endothelium.

Interaction between acetylcholine and antagonists of cromakalim

Figures 6a and b show original traces examining the effects of phentolamine (100 μM) and the subsequent addition of cromakalim and acetylcholine in a vessel with an intact endothelium. In this particular experiment phentolamine caused a large depolarization from −64 to −56 mV. While the effects of cromakalim (1 − 30 μM) were completely inhibited (Figure 6a), acetylcholine-induced hyperpolarization still occurred, although higher concentrations were required (Figure 6b). Further studies were performed examining acetylcholine concentration-effect curves constructed in the absence and presence of glibenclamide (3 μM), alinidine (30 μM) and phentolamine (30 and 100 μM). The tissues were equilibrated with the antagonists for 10 min before construction of the acetylcholine curve. In the absence of the antagonists acetylcholine caused a concentration-dependent hyperpolarization with an EC_{50} of approximately 0.03 μM (Figure 7). Phentolamine (30 μM) and alinidine (30 μM) caused an approximate 10 fold rightward shift in the curve (EC_{50} 0.3 μM) (Figure 7). There was no further shift in the curve when the phentolamine concentration was increased to 100 μM (Figure 7). Glibenclamide failed to affect the EC_{50} of acetylcholine although, due to its direct depolarizing effect, the resting membrane potential was less negative for any given concentration of acetylcholine (Figure 7).

Functional effects of cromakalim antagonists on responses to cromakalim and acetylcholine

The cromakalim antagonists were also tested for their ability to affect the relaxation responses to acetylcholine and cromakalim in mesenteric vessels precontracted with endothelin-1. The responses to cromakalim (0.1–30 μM) were shifted approximately 10 fold to the right in the presence of glibenclamide (3 μM), alinidine (30 μM) and phentolamine (30 μM) (Figure 8). Using acetylcholine as the vasorelaxant however we found gli-

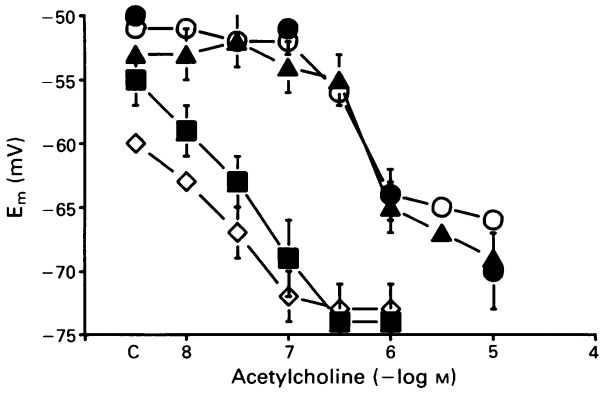

Figure 7 Mean concentration-effect curves for acetylcholine on membrane potential (E_m) in the rat small mesenteric artery. Curves were constructed in the absence (◇) and in the presence of glibenclamide (■, 3 μM), phentolamine (○, 30 and ●, 100 μM) and alinidine (▲, 30 μM). C is the control E_m just prior to the addition of any acetylcholine. Results are the mean for 3 to 6 separate experiments; s.e.mean shown by vertical bars. Each experiment was performed by constructing a concentration-effect curve to acetylcholine in the absence and then in the presence of the antagonist. The control curves were superimposable and consequently only one (n = 6) is included on the graph to aid clarity.

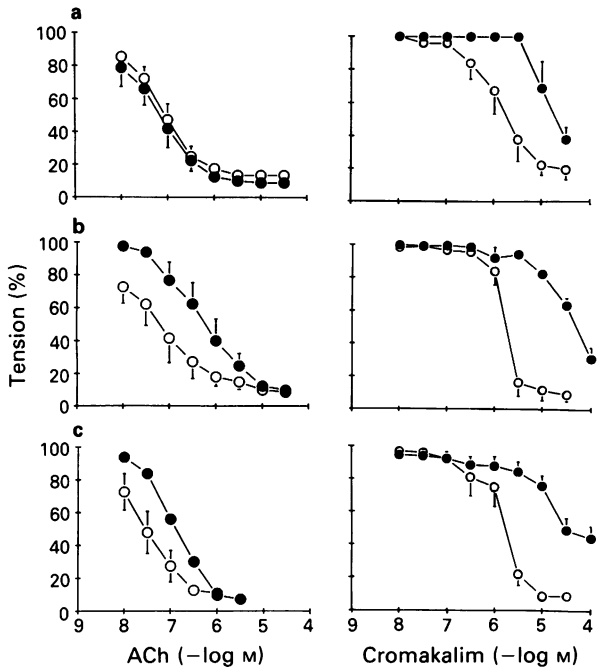

Figure 8 Mean concentration-effect curves constructed for acetylcholine (left panels) or cromakalim (right panels) in the rat small mesenteric artery. Tone was induced with a submaximal concentration of endothelin-1 (10–20 nM). Curves to cromakalim or acetylcholine were constructed in the absence (○) and in the presence (●) of glibenclamide (3 μM; a), phentolamine (100 μM; b) and alinidine (30 μM; c). Relaxation responses, expressed as a percentage of the tone induced by ET-1, are mean from 3 to 6 separate experiments; s.e.mean shown by vertical bars.

benclamide to be ineffective while alinidine and phentolamine caused a 5–10 fold shift to the right of the acetylcholine concentration-effect curve (Figure 8).

Anti-muscarinic effects of phentolamine and alinidine

Anti-muscarinic activity of phentolamine and alinidine were assessed in the pig large coronary artery. In this preparation acetylcholine caused a vasoconstrictor response with an EC_{50} of approximately 60 nM (Figure 9). The concentration-effect curve was shifted to the right (Figure 9) approximately 10 fold by phentolamine (100 μM) and 7 fold by alinidine (30 μM).

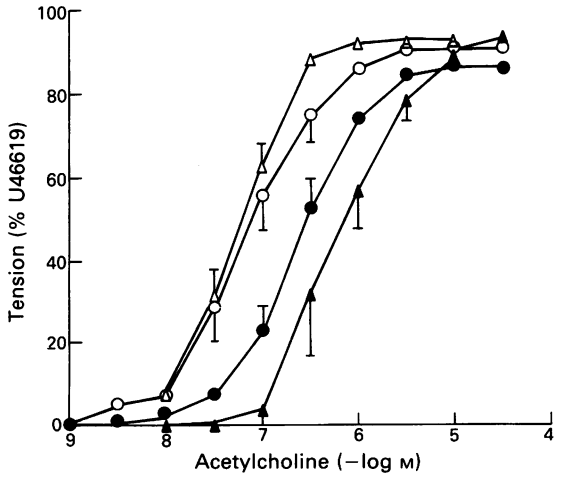

Figure 9 Mean concentration-effect curves for acetylcholine in the pig coronary artery. Curves were constructed for acetylcholine in the absence (open symbols) or in the presence (closed symbols) of phentolamine (▲, 100 μM) or alinidine (●, 30 μM). Contractile responses, expressed as a percentage of the response of the tissue to 11α,9α-epoxymethano-prostaglandin H_2 (U46619, 30 nM, a thromboxane-mimetic), are the mean of 4 to 5 separate experiments; s.e.mean shown by vertical bars.

Discussion

The main finding from this work is that acetylcholine-mediated hyperpolarization does not appear to involve the K^+ channel opened by cromakalim (ATP-sensitive K^+ channels). This result contrasts with that obtained by Standen and his colleagues (Standen *et al.*, 1989) who showed that acetylcholine-induced hyperpolarization in the rabbit middle cerebral artery involved the opening of ATP-sensitive K^+ channels since the response was reversed by glibenclamide. Using very similar microelectrode techniques however, we found that the hyperpolarization and functional vasorelaxant responses to acetylcholine were not susceptible to the effects of glibenclamide at concentrations that blocked the response to cromakalim. This result suggests that there may be two different hyperpolarizing substances released from the endothelium or possibly one substance which utilizes two different coupling mechanisms. In either case one process utilizes ATP-sensitive K^+ channels which are antagonized by glibenclamide (a mechanism activated in the rabbit middle cerebral artery) and at least one other process uses another, as yet unidentified, mechanism.

Whatever the mechanism of the observed hyperpolarization it would appear that it plays little role in the functional vasorelaxant response to acetylcholine since agents which block this response (e.g. methylene blue and haemoglobin) generally fail to affect the hyperpolarization (see Chen *et al.*, 1988; Nishiye *et al.*, 1989). These results have led workers to suggest that at least two factors are released by acetylcholine. EDRF (which is NO or related to NO) and EDHF (see Taylor & Weston, 1988). It is apparent from the literature and the results obtained in the present study that the electrophysiological effects (i.e. hyperpolarization) of acetylcholine are complex and possibly multifactorial. In some vessels such as the rabbit ear artery (Suzuki, 1988) and femoral artery (Huang *et al.*, 1988), rat pulmonary (Chen *et al.*, 1988) and canine mesenteric artery (Komori *et al.*, 1988) acetylcholine causes a small and transient (approximately 5 mV) hyperpolarization. In other studies such as those using the rat mesenteric artery (this study), cat middle cerebral artery (Brayden & Wellman, 1989), dog coronary artery (Chen *et al.*, 1989) and guinea-pig basilar artery (Nishiye *et al.*, 1989) the hyperpolarization can be as much as 25 mV and longer lasting. That the degree and type of hyperpolarization appear to fall into two categories may suggest differing underlying mechanisms. There is already some evidence to support this idea. For example, some reports have suggested that acetylcholine-induced hyperpolarization results from the activation of Na^+/K^+ ATPase since responses are antagonised by ouabain (Feletou & Vanhoutte, 1988; Brayden & Wellman, 1989) while others have shown ouabain to be ineffective (Suzuki, 1988; Chen *et al.*, 1989). The work of Standen and co-workers (1989) suggests that the ATP-sensitive K^+ channel is important but there are several other possibilities which may account for acetylcholine hyperpolarization of smooth muscle. First the work of Tare and co-workers (1990) has shown that authentic NO can indeed affect membrane potential in contrast to earlier studies which have suggested that this is not the case (Komori *et al.*, 1988; Huang *et al.*, 1988). Tare and co-workers (1990) showed that in the guinea-pig uterine artery, acetylcholine and NO both hyperpolarized membrane potential back to control levels in vessels activated with phenylephrine. This response is some what different from that seen in other studies in that the membrane potential did not hyperpolarize beyond that in the inactivated vessel, while most studies involving acetylcholine have shown a true hyperpolarization rather than just repolarization. For example in the present study, acetylcholine hyperpolarized the cell from the rat small mesenteric artery to the same absolute value (approximately −70 mV) irrespective of

whether the vessel was initially depolarized by a vasoconstrictor or not. In any case the observation that acetylcholine and NO can influence membrane potential suggests that the contribution of NO to changes in membrane potential should be examined more thoroughly. Lastly Busse and co-workers (1988) have shown that acetylcholine can also hyperpolarize endothelial cells directly. They raised the possibility that electrical coupling between the endothelial and underlying smooth muscle cell may well account for some of the hyperpolarizing response to acetylcholine. To summarize then, acetylcholine can produce varying degrees of hyperpolarization which depends on the vessel being studied. Such hyperpolarization can result from a number of mechanisms including Na^+/K^+ ATPase activation, K^+ channel opening and possibly other, as yet unidentified, mechanisms. Clearly this area of research requires much more work.

We also tested two other compounds, phentolamine and alinidine, which we have shown (McPherson & Angus, 1989) block the potassium channel opened by cromakalim. In contrast to the result that we obtained with glibenclamide, these two agents did shift the acetylcholine-mediated concentration-dependent vasorelaxant and hyperpolarization response curves to the right.

There may be a number of reasons for the shift in both functional and electrophysiological responses to acetylcholine observed with phentolamine and alinidine. First these agents may possess some anti-muscarinic activity which would affect the response to acetylcholine post-junctionally rather than by interfering with the actions of the intermediates released from the endothelium. We examined this possibility in the pig large coronary artery where acetylcholine causes a constrictor response through the direct activation of the smooth muscle. Use of this preparation avoided interpreting the complicated effects of acetylcholine on the rat small mesenteric artery. Responses to acetylcholine were antagonized by concentrations of alinidine (30 μM) and phentolamine (100 μM) which inhibited the functional and hyperpolarizing response in the rat mesenteric artery. In addition radioligand binding studies (Brunner & Kukovetz, 1988) have shown that alinidine displaces specific [^{3}H]-QNB binding in membranes prepared from guinea-pig heart muscle over the concentration-range of 10–100 μM. Lastly, in our previous studies assessing the selectivity of glibenclamide, phentolamine and alinidine in a number of vascular and non-vascular smooth muscle preparations (McPherson & Angus, 1990) we found that the relative potency of the antagonists in blocking the response to cromakalim did not vary significantly. As a consequence we think is unlikely that, in the rat small mesenteric artery, glibenclamide would not block a K^+ channel susceptible to the actions of phentolamine and alinidine. On the bases of these results we feel that phentolamine and alinidine are not appropriate tools for studying the involvement of K^+ channels in mediating the effects of acetylcholine.

Of greater interest was the finding that all three compounds (glibenclamide, phentolamine and alinidine) had a direct depolarizing effect on resting membrane potential. Previous studies in our laboratory (McPherson & Angus, 1989; 1990) have shown glibenclamide to be approximately 10–30 times more potent than phentolamine and alinidine in blocking the effects of the response to cromakalim. Glibenclamide is active at approximately 1 μM while phentolamine and alinidine are active at 30 μM. This relative potency coincides almost exactly with their ability to induce depolarizing responses in the rat small mesenteric artery. There may be several reasons for this phenomenon. Firstly it is possible that in this artery there is a basal release of a hyperpolarizing factor which tonically influences resting membrane potential. The basal release of EDRF has previously been considered (Moncada & Palmer, 1990) which prompted us to examine the endothelial-dependence of the response. However, in vessels which had the endothelium removed, the compounds were still able to depolarize to some extent. This result would exclude the basal release of an endothelium-derived factor but it does not exclude the release of such a factor from other sites in the blood vessel wall. Another possibility is that the K^+ channel is partially opened in the rat small mesenteric artery under the conditions in which we assessed membrane potential. It is possible that the three compounds, rather than being antagonists of the compounds that open the channel (i.e. occupy the receptor site but do not initiate a response), are actually 'inverse agonists' in that they actively close the K^+ channels if they are open. The interaction between these compounds and K^+-channel openers such as cromakalim and pinacidil is not then a matter of competitive but rather functional antagonism. If this idea proves to be correct then there is obviously some variation in the percentage of K^+ channels spontaneously open since in vessels such as the rat femoral artery (McPherson & Angus, 1989) and rabbit middle cerebral artery (Standen *et al.*, 1989) the compounds do not display direct depolarising effects on the resting membrane potential. Whatever the case may be, the results from this study show that, in the rat mesenteric artery, the activity of K^+ channels sensitive to glibenclamide, phentolamine and alinidine increase the negativity of the membrane potential by approximately 10 mV. Clearly much more work is required in this area.

In summary the results from this study using glibenclamide to block ATP-dependent K^+ channels suggest that this channel plays no role in the hyperpolarizing response to acetylcholine in the rat small mesenteric artery. This result contrasts with those obtained in the rabbit middle cerebral artery described by other workers (Standen *et al.*, 1989). Taken collectively, the results show that acetylcholine-induced hyperpolarization can result from a number of mechanisms, the physiological significance of which has yet to be determined.

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