Endothelium-dependent hyperpolarization in resting and depolarized mammary and coronary arteries of guinea-pigs

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1 The membrane potential responses in guinea-pig coronary and mammary arteries attributable to endothelium-derived nitric oxide (NO), prostaglandin (PG) and hyperpolarizing factor (EDHF), and to exogenous NO and the prostacyclin analogue, iloprost, were compared at rest and when depolarized with the thromboxane analogue, U46619.

2 In the coronary artery, stimulation of the endothelium with acetylcholine (ACh) evoked hyperpolarization attributable to NO and a PG with similar pD₂s at rest and in the presence of U46619. However, in depolarized tissues, the pD₂ of the response attributed to EDHF required a 10 fold lower concentration of ACh compared with at rest.

3 In the mammary artery, lower concentrations of ACh were required to evoke NO- and EDHF-dependent hyperpolarizations in depolarized mammary artery compared with at rest, while PG-dependent hyperpolarization did not occur until the concentration of ACh was increased some 10 fold both at rest and in U46619.

4 The smooth muscle of the coronary artery of guinea-pigs was some 4 fold more sensitive to exogenous NO and iloprost than was the mammary artery.

5 In conclusion, the membrane potential response in arteries at rest, that is, in the absence of constrictor, may be extrapolated to events in the presence of constrictor when NO and PG are under study. However, the sensitivity to ACh and the magnitude of the hyperpolarization attributed to EDHF obtained in tissues at rest may underestimate these parameters in depolarized tissues.

Keywords: Nitric oxide; prostacyclin; EDHF; iloprost; U46619; hyperpolarization; coronary artery; mammary artery

Abbreviations: ACh, acetylcholine; EDHF, endothelium-derived hyperpolarizing factor; Ilo, iloprost; Indo, indomethacin; L-NAME, N^ω-nitro L-arginine methylester; NO, nitric oxide; PG, prostaglandin; V_{max}, peak hyperpolarization amplitude

Introduction

The vascular endothelium is capable of releasing nitric oxide (NO), prostacyclin (PGI) and endothelium-derived hyperpolarizing factor (EDHF) which evoke hyperpolarization of the smooth muscle in a variety of vascular beds: NO, (Tare *et al.*, 1990; Garland & McPherson, 1992; Parkington *et al.*, 1993), PGI (Siegel *et al.*, 1987; Parkington *et al.*, 1993) and EDHF (Chen *et al.*, 1988; Feletou & Vanhoutte, 1988). Attempts to understand the role of hyperpolarization in relaxation of vascular smooth muscle has received considerable attention. However, in many studies hyperpolarization is not recorded simultaneously with relaxation. While relaxation is studied in precontracted arterial tissue, hyperpolarization is most commonly recorded in tissues at rest, in the absence of constrictors. Potassium channels are likely to be an important mechanism of endothelium-dependent hyperpolarization in many arterial beds (Nelson & Quayle, 1995). The open probability of some K⁺ channels is influenced by membrane potential, while others are sensitive to cytoplasmic Ca²⁺. Both membrane potential and cytoplasmic Ca²⁺ are commonly altered by constrictors.

It is well established that not all of the agents used to precontract arteries have the same effect on ionic conductances in vascular smooth muscle, an important consideration in view of the role of competing conductances in determining the ability to hyperpolarize. For example, α -adrenoceptor agonists

provide a substantial increase in calcium-activated chloride conductance in arterial smooth muscle (Amédée *et al.*, 1990), while the thromboxane A₂ analogue U46619 has been shown to inhibit Ca²⁺-activated K⁺ channels (Scornik & Toro, 1992). In rat mesenteric arteries that are depolarized and contracted with noradrenaline, stimulation of the endothelium causes relaxation and hyperpolarization that are resistant to blockade of NO synthesis, whereas in arteries that are depolarized and contracted with U46619 no hyperpolarization is observed (Plane & Garland, 1996).

The effectiveness of the agents released from the endothelium to relax an artery is pivotally dependent upon the ability of the smooth muscle to respond. This issue has been largely neglected, with few exceptions (Christie & Lewis, 1988). This omission may complicate interpretation of comparison between endothelium responsiveness in different arterial beds.

In the present study we compared the ability of endothelium-derived NO, PG and EDHF to hyperpolarize guinea-pig coronary and mammary arteries. The coronary artery has been studied in some detail in this laboratory and the mammary artery was included to broaden interpretation. The responsiveness of the smooth muscle of the two arteries to exogenous vasorelaxants was compared. Finally, the responses to endogenous and exogenous vasodilators were compared at rest and, in view of the focus on membrane hyperpolarization, under conditions where underlying membrane conductances were altered with U46619.

Some of these results have been presented previously (Hammarström *et al.*, 1991).

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Methods

Preparations

Guinea-pigs of either gender (300–700 g) were killed by decapitation. The mammary artery, just proximal to its major branch point within the mammary fat, and the main descending coronary artery, just prior to its entry into the myocardium, were removed. Ring segments, 1–2 mm in length, were mounted on a myograph (bath capacity 1 ml) (Mulvany & Halpern, 1977) using a pair of stainless steel wires (40 μ m in diameter) and continuously superfused with physiological solution (at 35°C) containing (mM): NaCl, 120; KCl, 5; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1; NaHCO₃, 25; glucose, 11, bubbled with O₂, 95% and CO₂, 5%, at a rate of 3.6 ml min⁻¹. The preparations were stretched to a tension equivalent to approximately 60 mmHg, the mean blood pressure of guinea-pigs. Conventional intracellular glass microelectrodes, filled with 1 M KCl and having resistances of around 100 M Ω , were used to impale the smooth muscle cells and record membrane potential (Parkington *et al.*, 1993). Isometric tension was recorded simultaneously *via* a force transducer (Kistler-Morse, DSC-6BE4-110) attached to one arm of the myograph. The data were recorded on chart (Linearcorder) and stored on video cassette using a digital data recorder for analysis.

Experiments

The endothelium was stimulated by injecting acetylcholine (ACh) straight into the perfusion line close to the tissue (1 s delay) for a period of 10 s. This method of stimulation allows the component of membrane hyperpolarization attributable to each of the vasorelaxants to be distinguished unambiguously, in contrast with the responses to more prolonged (1–2 min) applications (Parkington *et al.*, 1993; 1995). The exogenous vasorelaxants NO and iloprost, a stable analogue of prostacyclin, were also applied for 10 s. Injection of 5–100 μ l of saline (the range of volumes used when injecting agonists) had no effect on membrane potential or tension. The concentration of these agonists reached equilibrium within 3–5 s (see Parkington *et al.*, 1993). Concentration-response relationships using this method of applying ACh have been shown not to differ from the relationship obtained when ACh is added to the superfusate for 1 min (Hammarström *et al.*, 1995). NO synthesis was blocked using 5×10^{-5} M N^o-nitro-L-arginine methyl ester (L-NAME) and prostaglandin synthesis was blocked using 10^{-6} M indomethacin, both applied for 30–40 min prior to and during testing. In a pilot study of five coronary and five mammary arteries, increasing the concentration of L-NAME to 10^{-4} M and then to 10^{-3} M had no additional inhibitory effect compared with that of 5×10^{-5} M on the concentration-hyperpolarization curve in response to ACh. Similarly, increasing indomethacin to 10^{-5} M failed to produce an effect additional to 10^{-6} M. NO solution was made by transferring the appropriate volume of NO gas, using a gas tight micro-syringe, to a sealed vial containing 0.9% saline that had been de-oxygenated by bubbling with argon for 60 min.

Analysis of data

The amplitude (mV) of the hyperpolarization was measured from the level of the membrane potential prior to the application of vasorelaxant to the peak of the response. The maximum amplitude of the response to each agonist has been

designated as $V_{\max}$ according to convention. The sensitivity of the endothelium and smooth muscle was determined by converting the hyperpolarization to a percentage of the maximum hyperpolarization obtained in that particular preparation, whether the latter was due to an endogenously released relaxing factor or to exogenously applied vasorelaxant. A sigmoid curve was fitted to the data for each preparation, using the least-squares method, using the computer software package Prism (GraphPad Software, Inc.). The concentration of agonist which was effective in producing a response that was 50% of maximum (EC_{50}) and the Hill slope of the curve were determined for each tissue. The mean values of EC_{50} and the mean slope for 4–8 preparations were then used to generate a curve that was representative of all preparations in the group. pD_2 values ($-\log EC_{50}$) have been quoted throughout.

The statistic quoted with each mean is the standard error based on the number of animals tested (n). A Student's t -test was used to test for significance which was accepted at $P < 0.05$.

Drugs

The following drugs were used: ACh (Merck), L-NAME and indomethacin (Sigma, U.S.A.), U46619 and PGE₂ (Cayman Chemical Co.), Iloprost (a gift from Schering, Germany), NO (Matheson Gas Products Inc., U.S.A.).

Results

When stretched to the equivalent of approximately 60 mmHg, the effective diameter of preparations of mammary artery was 476 ± 16 μ m ($n = 12$), which was not significantly different from the value of 519 ± 26 μ m ($n = 13$) for coronary artery. The resting membrane potential of the smooth muscle cells of the mammary artery was -57 ± 1 mV ($n = 20$), which was not significantly different from the -55 ± 1 mV ($n = 12$) recorded in the coronary artery.

Hyperpolarization evoked by acetylcholine at rest

At rest, ACh evoked hyperpolarization in the mammary artery that consisted of an initial transient component, followed by a slow component, similar to that reported previously for the coronary artery in this laboratory (Parkington *et al.*, 1993). The amplitudes of both components were concentration dependent. Either L-NAME (5×10^{-5} M) or indomethacin (10^{-6} M) alone reduced the amplitude of the slow component in both arteries. In the presence of both blockers the slow component was completely abolished, and hence can be attributed to the combined actions of NO and PG in both arteries. The transient component remaining in the presence of both L-NAME and indomethacin has been attributed to the actions of EDHF (Komori & Suzuki, 1987; Taylor & Weston, 1988; Parkington *et al.*, 1993).

L-NAME and indomethacin together caused significant depolarization, from -55 ± 1 to -45 ± 1 mV ($n = 6$), in the coronary artery. In contrast, these blockers did not significantly change the resting membrane potential in the mammary artery (from -54 ± 1 to -51 ± 2 mV, $n = 6$). Furthermore, the membrane potential of the smooth muscle cells of the mammary artery were unchanged following removal of the endothelium (-54 ± 1 mV, $n = 6$), and this is in contrast with previous observations in coronary artery (Parkington *et al.*, 1993).

Endogenous prostaglandin

ACh was applied at rest in the presence of L-NAME to abolish a contribution by NO to the slow component, allowing examination of the component of the hyperpolarization evoked by endothelium-derived PG (Figure 1A, and B). In the presence of L-NAME, the resting membrane potential in the coronary artery (-51 ± 2 mV, $n=6$) was less negative than that of the mammary artery (-58 ± 2 mV, $n=8$). The slow component of the hyperpolarization increased in amplitude as the concentration of ACh was increased, reaching a maximum of 15 ± 2 mV ($n=6$) in the coronary artery and a significantly

smaller value of 10 ± 1 mV ($n=8$) in the mammary artery. However, the maximum absolute level of membrane potential reached during the slow component of hyperpolarization attributable to a PG was not different in the two arteries (to -68 ± 1 mV, $n=6$, in the coronary and to -71 ± 3 mV, $n=8$, in the mammary arteries). The ACh-induced hyperpolarization attributable to a PG in the coronary artery had a pD_2 of 6.66 ± 0.12 ($n=6$) and this was significantly larger than the pD_2 of 5.25 ± 0.14 ($n=7$) for the mammary artery (Figure 1C, Table 1).

Exogenous prostaglandin

Prostacyclin is the main PG released from the guinea-pig coronary artery by ACh (Parkington *et al.*, 1993) and is also likely to be the principal vasodilator PG in the mammary artery since PGE₂ evoked only a small (0–2 mV) hyperpolarization in the latter ($n=3$, data not shown). The effects of the stable analogue of prostacyclin, iloprost, on membrane potential was tested, also in the presence of L-NAME. Iloprost evoked concentration-dependent hyperpolarization (Figure 2) with a pD_2 of 7.95 ± 0.09 ($n=5$) in the coronary artery and a significantly smaller pD_2 of 7.31 ± 0.14 ($n=7$) in the mammary artery (Figure 2, Table 1). V_{max} for the hyperpolarization was significantly greater in the coronary (17 ± 1 mV, $n=6$) than in the mammary (12 ± 1 mV, $n=8$) artery. However, the maximum level of the membrane potential reached during the response to iloprost was not different in the two arteries (to

Figure 1 In the presence of L-NAME, ACh evoked hyperpolarization in the mammary (A) and coronary (B) arteries that consisted of an initial transient component followed by a slow, more sustained component. The amplitude of the slow component was expressed as a percentage of the maximum hyperpolarization in each respective tissue. C shows the mean $\pm$ s.e. mean and the curves generated from the mean EC_{50} and mean Hill slope for responses from six coronary and seven mammary arteries.

Figure 2 The hyperpolarizations evoked by iloprost, in the presence of L-NAME, were expressed as a percentage of the maximum hyperpolarization in each tissue (mean $\pm$ s.e. mean). Curves were generated from the mean EC_{50} and mean Hill slope for responses from five coronary and seven mammary arteries.

Table 1 Sensitivity to vasorelaxants at rest

Source of factor	Artery	L-NAME (PG) ($pD_2 \pm$ s.e.)	Indomethacin (NO) ($pD_2 \pm$ s.e.)	L-NAME+Indo (EDHF) ($pD_2 \pm$ s.e.)
Endogenous	Coronary (n)	6.66 ± 0.12 (6)	6.83 ± 0.15 (5)	6.5 ± 0.2 (6)
	Mammary (n)	5.25 ± 0.14 (7)	6.27 ± 0.18 (5)	6.4 ± 0.1 (5)
Exogenous	Coronary (n)	7.95 ± 0.09 (5)	5.56 ± 0.11 (4)	
	Mammary (n)	7.31 ± 0.14 (7)	5.14 ± 0.08 (5)	

ACh evoked hyperpolarization that could be attributed to the release of endogenous PG, NO and EDHF in arteries at rest. Iloprost was used as the exogenous PG. The number of animals in each group is represented by *n*.

-68 ± 3 mV, $n=6$ in the coronary and to -72 ± 4 mV, $n=7$ in the mammary).

Endogenous NO

Indomethacin was used to block the release of PGs from the endothelium, and hence isolate the slow component of the hyperpolarization attributable to the actions of endogenous NO alone. In the presence of this blocker the resting membrane potential in the two arteries was not different (-53 ± 3 mV, $n=5$ in the coronary and -55 ± 1 mV, $n=5$ in the mammary). In these experiments L-NAME was applied at the end of the experiment and a range of concentrations of ACh applied again in order to unequivocally confirm the response to NO.

The slow component of hyperpolarization elicited by ACh in the presence of indomethacin had a pD_2 of 6.83 ± 0.15 ($n=5$) in the coronary artery and a pD_2 of 6.27 ± 0.18 ($n=5$) in the mammary artery (Figure 3 and Table 1), values that were significantly different. The V_{max} of the response attributable to NO had similar values in both preparations (13 ± 3 mV, $n=6$, in coronary and 10 ± 1 mV, $n=5$, in mammary artery).

Exogenous NO

Since the responses to endogenous NO were obtained in the presence of indomethacin, that blocker was also present when examining the effects of exogenous NO (Figure 4). The hyperpolarizations evoked by exogenous NO had similar maximum amplitudes in the coronary (11 ± 1 mV, $n=7$) and the mammary (9 ± 1 mV, $n=5$) arteries. The pD_2 of 5.56 ± 0.11 ($n=4$) in the coronary artery was significantly different from the value of 5.14 ± 0.08 ($n=5$) in the mammary artery (Figure 4, Table 1). However, the level of membrane potential reached was the same for both arteries (-64 ± 3 mV, $n=5$ for coronary; -63 ± 2 mV, $n=4$ for mammary).

Endothelium-derived hyperpolarizing factor (EDHF)

The initial transient component of the hyperpolarization in response to ACh, which was resistant to both L-NAME and indomethacin, was attributed to EDHF. In the presence of both blockers, to avoid contamination with endogenous NO and PG, the peak amplitude of the transient component increased progressively in both arteries as the concentration of

ACh was increased, reaching a maximum of 24 ± 2 mV ($n=5$) in the coronary artery. This was significantly larger than the 8 ± 1 mV ($n=5$) evoked in the mammary artery (Figure 5). Furthermore, the absolute maximum level of membrane potential reached at the peak of the transient component was significantly more hyperpolarized in the coronary artery (to -72 ± 5 mV, $n=5$) than in the mammary artery (to -57 ± 3 mV, $n=6$) (Figure 5). The pD_2 for the transient component was 6.5 ± 0.2 ($n=6$) in the coronary artery and 6.4 ± 0.1 ($n=5$) in the mammary artery (Figure 5, Table 1). These values were not significantly different.

Hyperpolarization in the presence of U46619

The concentration of U46619 was varied (10^{-8} – 10^{-7} M) in order to achieve a submaximal depolarization that was similar in both arteries (to -38 ± 1 mV). The depolarization was often accompanied by the firing of action potentials, as shown previously for coronary artery (Parkington *et al.*, 1995) and an example in mammary artery is shown in Figure 6. ACh evoked concentration-dependent, biphasic hyperpolarizations, similar in form to those observed in tissues at rest (Figure 6). Application of L-NAME or indomethacin in the presence of U46619 evoked an additional depolarization of approximately

Figure 4 The hyperpolarizations evoked by NO, in the presence of indomethacin, were expressed as a percentage of the maximum hyperpolarization in each tissue (mean $\pm$ s.e. mean). Curves were generated from the mean EC_{50} and the mean slope from four coronary and five mammary arteries.

Figure 3 The slow component of the hyperpolarization evoked by ACh, in the presence of indomethacin, was expressed as a percentage of the maximum hyperpolarization in each tissue (mean $\pm$ s.e. mean). Curves were generated from the mean EC_{50} and the mean Hill slope for responses from five coronary and five mammary arteries.

Figure 5 The level of membrane potential attained during the transient hyperpolarization evoked by ACh, in the presence of L-NAME and indomethacin, was plotted for six coronary and five mammary arteries.

5 mV in both arteries. In order to avoid a change in membrane potential the concentration of U46619 was reduced, for example, from 3×10^{-8} M to 2×10^{-8} M or from 7×10^{-8} M to 5×10^{-8} M, which maintained the starting level of membrane potential constant for all treatments.

Endogenous prostaglandin and NO in the presence of U46619

In the presence of L-NAME to block NO production, the slow component of hyperpolarization in response to ACh had a pD_2 of 6.54 ± 0.16 ($n=5$) in the coronary which was significantly different from the pD_2 of 5.59 ± 0.07 ($n=5$) in the mammary artery (Figure 7, Table 2). In the presence of indomethacin to block PG production, the pD_2 s for the slow component evoked by ACh were similar in the coronary (6.80 ± 0.10 , $n=5$) compared with the mammary (6.74 ± 0.29 , $n=5$) (Figure 7, Table 2). The combined application of L-NAME and indomethacin abolished the slow component completely (solid squares in Figure 7).

V_{max} for the hyperpolarization attributable to NO and PG was similar in the coronary (18 ± 4 and 20 ± 4 mV, respectively, $n=5$ for each) and in the mammary (17 ± 3 and 15 ± 4 mV, respectively, $n=5$ for each tissue) artery. Since the starting membrane potential was the same, the maximum level of the

Table 2 Sensitivity to vasorelaxants in U46619

Artery	L-NAME (PG) ($pD_2 \pm s.e.$)	Indomethacin (NO) ($pD_2 \pm s.e.$)	L-NAME + Indo (EDHF) ($pD_2 \pm s.e.$)
Coronary (n)	6.54 ± 0.16 (5)	6.80 ± 0.10 (5)	7.77 ± 0.09 (10)
Mammary (n)	5.59 ± 0.07 (5)	6.74 ± 0.29 (5)	6.93 ± 0.05 (10)

ACh evoked hyperpolarization that could be attributed to the release of endogenous PG, NO and EDHF in arteries depolarized using U46619. The number of animals in each group is represented by n .

Figure 6 In the mammary artery ACh evoked hyperpolarization (upper panel) and relaxation (lower panel) in control solution. Indomethacin reduced the duration of the response. In the presence of indomethacin plus L-NAME the responses were brief: the slow component was abolished leaving the transient component that has been attributed to EDHF. U46619 was present throughout. All responses are from a continuous impalement in the same cell.

Figure 7 Effect of L-NAME, indomethacin and L-NAME plus indomethacin on the peak levels of the slow ($n=5$ in each group) and fast ($n=10$) components of hyperpolarization in response to ACh in coronary and mammary arteries in the presence of U46619.

membrane potential reached during the hyperpolarizations were not different (-51 to -59 mV).

EDHF in the presence of U46619

The initial fast component (in the presence of L-NAME and indomethacin) of the hyperpolarization in response to ACh, and attributable to EDHF, had a pD_2 of 7.77 ± 0.09 ($n = 10$) in the coronary artery and this was significantly different from the pD_2 for the mammary artery (6.93 ± 0.05 , $n = 10$) (Figure 7, Table 2).

V_{max} for the hyperpolarization was significantly greater in the coronary (37 ± 1 mV, $n = 5$) than in the mammary artery (20 ± 4 mV, $n = 5$). Hence, the maximum level of the membrane potential reached during the initial hyperpolarization was greater in the coronary (-74 ± 1 mV, $n = 5$) compared with in the mammary artery (to -57 ± 3 mV, $n = 5$).

Direct effects of ACh on the smooth muscle

We have previously shown that high concentrations of ACh evoke only a small direct depolarization ($1-3$ mV) of the smooth muscle in preparations of coronary artery denuded of endothelium (Parkington *et al.*, 1993). Following removal of the endothelium from preparations of mammary artery, ACh failed to evoke hyperpolarization. ACh, $> 10^{-6}$ M applied to the superfusate for 1 min, had only a weak direct depolarizing effect (1.5 ± 0.4 mV) on the smooth muscle in 5 of 6 preparations, with no change in membrane potential in the remaining tissue. The depolarization was accompanied by a small (less than 3% of maximal) transient contraction (data not shown). The integrity of the smooth muscle was confirmed by the ability of nitroprusside or iloprost to hyperpolarize denuded preparations (data not shown).

Since L-NAME and indomethacin were used extensively in this study, their possible effects on the responses to exogenous NO and iloprost were tested. In five preparations of coronary artery L-NAME had no effect on the hyperpolarization evoked by iloprost or exogenous NO (data not shown). Likewise, indomethacin had no effect on the response to iloprost or exogenous NO in five other tissues (data not shown). The hyperpolarization evoked by either iloprost or exogenous NO was unaltered by removal of the endothelium.

Discussion

The results of the present study demonstrate that the ability of ACh to evoke L-NAME and indomethacin sensitive hyperpolarization is similar in coronary artery at rest as it is when depolarized with U46619. This contrasts with observations in the presence of both these blockers, in which the coronary is more than an order of magnitude more sensitive to ACh when depolarized compared with at rest. The mammary artery appears to be half an order of magnitude more sensitive to ACh when depolarized with U46619, irrespective of the presence of L-NAME, indomethacin or both blockers. The results presented here complement the study of Plane & Garland (1996) which demonstrated a difference in endothelium-dependent hyperpolarization according to the nature of the constrictor.

The peak levels of membrane potential attained during the hyperpolarization attributable to NO and PG were more negative at rest in both arteries. This presumably results from the inability of the conductance underlying the hyperpolarization to fully compete against the additional conductances

associated with the U46619-induced depolarization. In contrast, the hyperpolarization attributed to EDHF attained the same absolute level at rest and in depolarized tissues and this is consistent with a more robust conductance underlying the response to EDHF. The nature of the conductance involved has not been resolved definitively. However, a channel that may be similar to the large conductance, Ca^{2+} -activated K^+ channel has been implicated (Hashitani & Suzuki, 1997; Zygmunt *et al.*, 1997).

The pD_2 s for hyperpolarization recorded here are of similar order of magnitude for relaxation reported previously by us (Parkington *et al.*, 1995) and by others (Keef & Bowen, 1989; Bruch *et al.*, 1997). The coronary artery was 4 fold more sensitive to the ACh-evoked hyperpolarization attributable to NO than was the mammary artery. This could be accounted for entirely by the similar lower sensitivity of the mammary smooth muscle to exogenous NO. Christie & Lewis (1988) have also reported a greater sensitivity to nitroprusside of pig coronary artery compared with aorta.

Stimulation of the coronary endothelium induced considerable indomethacin sensitive hyperpolarization, and the smooth muscle hyperpolarized in response to the prostacyclin mimetic, iloprost. This confirms previous observations on this vessel in which relaxation was recorded (Lee *et al.*, 1990, Parkington *et al.*, 1995). The present study revealed that the mammary artery does not behave similarly. While the smooth muscle was some 4 fold less sensitive to exogenous prostacyclin in the mammary compared with the coronary artery, the mammary appeared to be some 26 fold less sensitive to ACh-induced hyperpolarization attributable to PG than the coronary artery. Thus, there remained a 6 fold lower sensitivity of the mammary endothelium with respect to prostacyclin release compared with that of the coronary artery. Lee *et al.* (1990) also found that the guinea-pig aorta was not relaxed by endothelium-derived PG. It is interesting that the hyperpolarization attributable to endogenous PG is reduced to around one third of normal in human coronary artery with atherosclerosis (Siegel *et al.*, 1993), while the literature abounds with reports of other arterial beds in which endothelium-dependent relaxation is resistant to indomethacin. Thus, it may be that prostacyclin is especially important in the physiology of the coronary circulation.

In the coronary artery, the concentration of ACh required to evoke the release of all three endogenous vasorelaxants, NO, PG and EDHF was similar in arteries at rest. Using different arteries and species, several studies have indicated that the muscarinic receptor responsible for the production of NO (Adeagbo & Triggle, 1993), PG (Jaiswal & Malik, 1990) and EDHF (Adeagbo & Triggle, 1993; Hammarström *et al.*, 1995) is likely to be the M_3 sub-type. Hence, the lesser ability of the mammary endothelium to produce PG is unlikely to be a consequence of a lower density of muscarinic receptors. Possible factors contributing to the reduced response of the mammary endothelium include the availability of arachidonic acid or prostacyclin, or the shunting of the pathway towards the production of thromboxanes, or *via* the lipoxygenase or cytochrome P450-like enzyme pathways.

The responses of the two arteries to exogenous NO and prostacyclin were remarkably similar. The extent to which the smooth muscle of the coronary artery was more sensitive than that of the mammary was similar for both vasodilators, 3–4 fold. The maximum amplitudes of the hyperpolarizations were similar in both arteries.

The maximum amplitude of the hyperpolarization attributable to EDHF and the level of membrane potential reached was much larger in the coronary compared with the mammary

artery. This result was complicated by the different levels of the membrane potential in these tissues at rest, a problem that was overcome in the presence of U46619, which induced depolarization to the same value in both arteries. The result may reflect a greater number of receptors for EDHF in the coronary artery, enhanced post-receptor mechanisms or the nature and/or density of K^+ channels on the smooth muscle cells. Vascular smooth muscle cells possess a variety of different K^+ channels and considerable heterogeneity exists between different vascular beds (Nelson & Quayle, 1995; Garland *et al.*, 1995).

When depolarized and constricted with U46619, the mammary artery was more sensitive to all three dilators, NO, PG and EDHF. This could reflect an increase in the driving force for K^+ . The situation is more complicated in the coronary artery. The sensitivity of the latter to NO and PG was unchanged when depolarized and constricted with U46619 compared with at rest. This could reflect a significant conductance in response to NO and PG at rest, which is capable of withstanding the additional conductances evoked by U46619 in the coronary. The enhanced sensitivity to EDHF in the presence of U46619 could reflect an enhancement by U46619 of EDHF release. Another possibility is that U46619, or perhaps the associated increase in cytoplasmic Ca^{2+} , enhances the availability of the channel involved in the EDHF hyperpolarization. The differences in the effect of depolarization in the EDHF responses in the coronary and mammary arteries raises the possibility that the identity of EDHF may not be the same in the two arteries.

Removal of the endothelium was without effect on either the resting membrane potential or on resting tone in the

mammary artery, in contrast with observations in coronary artery. Likewise, blockers of NO and PG synthesis were also without effect on these parameters in the mammary, in contrast with the coronary artery. The reasons for this are unclear but it suggests that at least some of the conflicting reports in the literature may be due to inherent differences between arteries.

In conclusion, the relatively clear-cut nature of the observations supports a role for measurement of membrane potential in the assessment of the role of hyperpolarization in mediating vasorelaxation. The recording of membrane potential at rest may be a reliable indicator of events over a wider range of conditions when dealing with the response to NO and PG. However, the arteries are likely to reveal the EDHF component of hyperpolarization more readily in depolarized tissues than at rest. The study of membrane potential alone to probe endothelial function can provide an alternative perspective on the possibilities of differential responsiveness to vasorelaxants uncomplicated by constrictor agents and elevated extracellular K^+ , which can introduce variability in their own right according to agent, concentration and arterial bed. The study provides the basis from which to introduce some of these variables, one at a time and in a controlled manner and in this way facilitate interpretation of the observed differences between blood vessels.

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