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# Role of gap junctions in the responses to EDHF in rat and guinea-pig small arteries

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**1** In guinea-pig internal carotid arteries with an intact endothelium, acetylcholine (10 $\mu$M) and levcromakalim (10 $\mu$M) each hyperpolarized the smooth muscle whereas a 5 mM elevation of extracellular $K^+$ was without effect.

**2** Incubation of the carotid artery with the gap junction inhibitors carbenoxolone (100 $\mu$M) or gap 27 (500 $\mu$M) essentially abolished the hyperpolarization to acetylcholine but it was without effect on that to levcromakalim. Carbenoxolone had no effect on the acetylcholine-induced endothelial cell hyperpolarization but inhibited the smooth muscle hyperpolarization induced by the endothelial cell $K^+$ channel opener, 1-ethyl-2-benzimidazolinone (600 $\mu$M).

**3** In rat hepatic and mesenteric arteries with endothelium, carbenoxolone (100 or 500 $\mu$M) depolarized the smooth muscle but did not modify hyperpolarizations induced by KCl or levcromakalim. In the mesenteric (but not the hepatic) artery, the acetylcholine-induced hyperpolarization was inhibited by carbenoxolone.

**4** Phenylephrine (1 $\mu$M) depolarized the smooth muscle cells of intact hepatic and mesenteric arteries, an effect enhanced by carbenoxolone. Gap 27 did not have a depolarizing action. In the presence of phenylephrine, acetylcholine-induced hyperpolarization of both hepatic and mesenteric artery myocytes was partially inhibited by each of the gap junction inhibitors.

**5** Collectively, the data suggest that gap junctions play some role in the EDHF (endothelium-derived hyperpolarizing factor) response in rat hepatic and mesenteric arteries. However, in the guinea-pig internal carotid artery, electrotonic propagation of endothelial cell hyperpolarizations *via* gap junctions may be the sole mechanism underlying the response previously attributed to EDHF.

**Keywords:** EDHF; gap junctions; gap 27; carbenoxolone; carotid artery; mesenteric artery; hepatic artery; endothelial cell; 1-EBIO

**Abbreviations:** EDHF, endothelium-derived hyperpolarizing factor; 1-EBIO, 1-ethyl-2-benzimidazolinone

## Introduction

In many small arterioles, acetylcholine stimulates the release of endothelium-derived hyperpolarizing factor (EDHF: Garland *et al*., 1995; Mombouli & Vanhoutte, 1997; Edwards & Weston, 1998). In rat mesenteric and hepatic arteries, Edwards *et al*. (1998) showed that a small increase in the extracellular $K^+$ concentration (up to 10 mM) could mimic the endothelium-dependent hyperpolarizations and that the combination of barium and ouabain antagonized both acetylcholine and $K^+$. The authors concluded that EDHF was $K^+$ which effluxed from endothelial cells and then hyperpolarized the smooth muscle by opening inwardly-rectifying potassium channels (sensitive to barium) and activating a $Na^+/K^+$-ATPase (sensitive to ouabain).

However, EDHF-induced hyperpolarizations of the guinea-pig internal carotid artery were not inhibited by a combination of barium and ouabain (Quignard *et al*., 1999). Furthermore, increasing extracellular $K^+$ by up to 10 mM failed to relax and hyperpolarize this preparation (Quignard *et al*., 1999), observations which contrast with the findings in the rat hepatic and mesenteric arteries (Edwards *et al*., 1998). A consistent observation in all these arteries was, however, the ability of the toxin mixture, apamin + charybdotoxin, to antagonize EDHF (Corriu *et al*., 1996; Edwards *et al*., 1998; Quignard *et al*., 1999).

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In some vessels it is possible that gap junctions transfer endothelial cell hyperpolarization (resulting from the activation of charybdotoxin- and apamin-sensitive $K^+$ channels on the endothelium) directly to the surrounding vascular smooth muscle (Kühberger *et al*., 1994; Chaytor *et al*., 1997; 1998; Dora *et al*., 1999; Yamamoto *et al*., 1998; 1999). Such an explanation would be consistent both with the universal toxin-sensitivity of the response and with the lack of effect of $K^+$ reported by Quignard *et al*. (1999).

In the present study, the effects of the gap junction inhibitors carbenoxolone and gap 27 have been investigated in arteries which are sensitive (rat hepatic and mesenteric) and insensitive (guinea-pig internal carotid) to elevation of extracellular $K^+$. Gap junctions are formed between cells by the docking of connexins, a process which is thought to involve their extracellular loops (see Brink, 1998). Gap 27, first characterized by Chaytor *et al*. (1997), is identical in sequence to a portion of an extracellular loop of a connexin designated Cx43. This is present in both vascular endothelial and smooth muscle cells (Christ *et al*., 1996) and it has been proposed that gap 27 may interfere with both gap junction formation and stability (Chaytor *et al*., 1998). The second inhibitor utilized in the present study, carbenoxolone, is a water-soluble salt of 18$\beta$-glycyrrhetinic acid. 18$\beta$-glycyrrhetinic acid reduces gap junction connections by a mechanism which is probably related to connexin dephosphorylation (Guan *et al*., 1996).

## Methods

Experiments were performed on hepatic and mesenteric arteries dissected from male Sprague-Dawley rats (150–200 g) and on internal carotid arteries from Duncan-Hartley guinea-pigs (400–480 g) previously killed by stunning and cervical dislocation.

### *Micro-electrode experiments*

Intact vessels were pinned to the Sylgard base of a heated bath (volume 10 ml) and superfused (10 ml $min^{-1}$), at 37°C, with Krebs solution containing 300 $\mu$M $N^G$-nitro-L-arginine and 10 $\mu$M indomethacin and gassed with 95% $O_2$/5% $CO_2$. Smooth muscle cells were impaled from the adventitial side, and endothelial cells from the intimal side, using micro-electrodes filled with 3 M KCl (resistance 40–80 MΩ). Acetylcholine, KCl, 1-ethyl-2-benzimidazolinone (1-EBIO) and levcromakalim were added as bolus injections directly into the bath in quantities calculated to give the final concentrations indicated. This occurred within 10 s after which the drug concentrations decayed to approximately 5% of their maximum value within 1 min.

Carbenoxolone was dissolved (in distilled water) immediately before use and added to the reservoir of Krebs superfusing the bath. To investigate the effects of gap 27, tissues were first pre-incubated at 37°C with the peptide for 60 min prior to the recordings and experiments were usually performed in the absence of the inhibitor (i.e. during its washout). However, one series of experiments was performed in the continued presence of gap 27 after the 60 min pre-incubation. Where appropriate, the preparations were exposed to phenylephrine for at least 20 min before as well as during the recordings. Experiments were performed using a conventional high impedance amplifier (Intra 767; WPI Instruments). Any 50 Hz interference at the amplifier output was selectively removed using an active processing circuit (Humbug; Digitimer) after which the signals were digitized and analysed using a MacLab system (AD Instruments).

### *Drugs and solutions*

Krebs solution comprised (mM): $Na^+$ 143, $K^+$ 4.6, $Ca^{2+}$ 2.5, $Mg^{2+}$ 1.2, $Cl^-$ 126.4, $H_2PO_4^-$ 1.2, $SO_4^-$ 1.2, $HCO_3^-$ 25, glucose 11.1 and was bubbled with 95% $O_2$ and 5% $CO_2$.

The following substances were used: acetylcholine chloride, carbenoxolone, 1-EBIO (1-ethyl-2-benzimidazolinone; Aldrich), gap 27 (amino acid sequence SRPTEKTIFII, purity >99%, synthesized by Severn Biotech), indomethacin, levcromakalim (SmithKline Beecham) and phenylephrine hydrochloride. Unless otherwise stated, all compounds were obtained from Sigma.

### *Statistics*

Students *t*-test (paired or unpaired as appropriate) was used to assess the probability that differences between mean values had arisen by chance.

## Results

### *Guinea-pig carotid artery*

In guinea-pig internal carotid arteries with an intact endothelium, acetylcholine and levcromakalim each induced hyperpolarizations of the smooth muscle whereas a 5 mM elevation of extracellular $K^+$ was essentially without effect (Figure 1). Incubation of the preparations with 100 $\mu$M carbenoxolone produced a small depolarization (4.0±1.3 mV, $n=5$) and almost abolished the response to acetylcholine (Figure 1). However, when endothelial cells were impaled, although the membrane potential was slightly depolarized ($P<0.05$), the hyperpolarization induced by acetylcholine was unaffected by 100 $\mu$M carbenoxolone (membrane potential: control −57.3±0.5 mV, $n=5$, carbenoxolone −59.1±0.3 mV, $n=4$; acetylcholine-induced hyperpolarization: control 20.5±0.6 mV, carbenoxolone 19.4±0.3 mV, $n=4$). The hyperpolarization induced by levcromakalim in the smooth muscle was similar in the absence or presence of carbenoxolone (Figure 1).

Following earlier studies (Warner *et al*., 1995; Chaytor *et al*., 1998; Dora *et al*., 1999), we also used a peptide inhibitor of gap junctions. Pre-incubation of arteries with 500 $\mu$M gap 27 for 1 h had no effect on the resting membrane potential or on the responses to levcromakalim (Figure 2). However, gap 27 essentially abolished the hyperpolarizations to 10 $\mu$M acetylcholine and to 600 $\mu$M 1-EBIO (Figure 2). Since in this series of experiments the effects of gap 27 were not investigated in the continued presence of the peptide (see Methods), the exposure to acetylcholine was repeated to determine the extent of washout during the course of the experiment. These experiments revealed a small, but significant, reversal of the inhibitory effects of gap 27 (Figure 2).

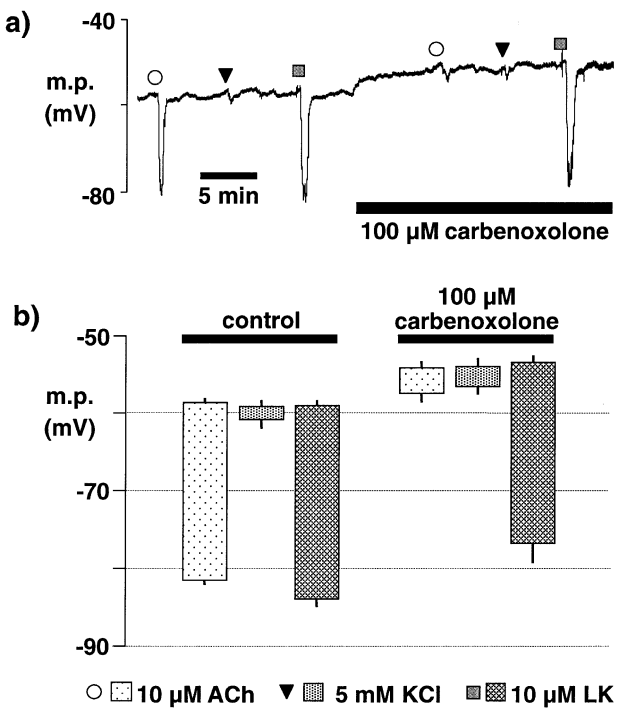


**Figure 1** Effects of carbenoxolone on hyperpolarizations induced by acetylcholine (ACh), potassium chloride (KCl) and levcromakalim (LK) in smooth muscle cells of guinea-pig intact internal carotid arteries. (a) Both acetylcholine and levcromakalim hyperpolarized the vessel whereas KCl had virtually no effect. On exposure to carbenoxolone, the muscle cells depolarized and responses to acetylcholine were almost abolished while those to levcromakalim were unaffected. Data from five separate experiments are shown graphically in (b) in which the columns represent the membrane potential (m.p.)±s.e.mean before and after exposure to each agent in the absence or presence of carbenoxolone as indicated.

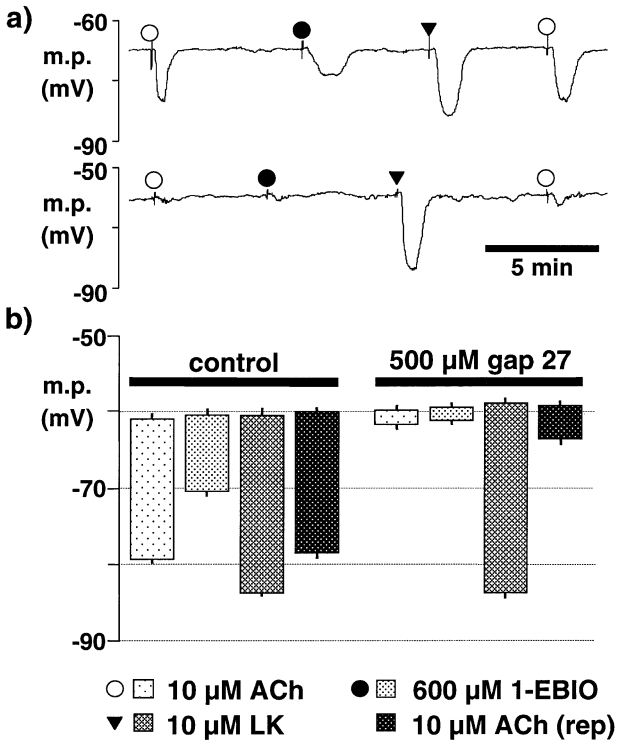


**Figure 2** Effects of gap 27 on hyperpolarizations induced by acetylcholine (ACh), 1-EBIO and levcromakalim (LK) in smooth muscle cells of guinea-pig intact internal carotid arteries. (a) The upper trace from a control vessel shows that acetylcholine, 1-EBIO and levcromakalim each produced smooth muscle hyperpolarization and that a second exposure to acetylcholine was as effective as the first. After 60 min incubation of the contralateral carotid artery in Krebs solution containing 500 $\mu$M gap 27, acetylcholine and 1-EBIO had little effect whereas a substantial hyperpolarization to levcromakalim was always observed. The second acetylcholine-induced hyperpolarization (b, rep) was always larger than the first. Data from four separate experiments are shown graphically in (b) in which the columns represent the membrane potential (m.p.)$\pm$s.e.mean in control vessels and in those exposed to gap 27 for 60 min as indicated.

### *Rat hepatic and mesenteric arteries*

Carbenoxolone (100 $\mu$M) depolarized hepatic artery smooth muscle cells by $9.2\pm0.8$ mV ($n=4$) in the absence of the endothelium and by $8.8\pm0.9$ mV ($n=4$) in intact vessels. Despite the depolarization, the magnitude of the hyperpolarizations induced by 10 $\mu$M acetylcholine (control, $26.7\pm1.1$ mV; cabenoxolone, $26.9\pm1.1$ mV, $n=4$), 5 mM KCl (control, $18.9\pm1.5$ mV; carbenoxolone, $21.2\pm0.8$ mV, $n=4$) and 10 $\mu$M levcromakalim (control, $29.9\pm0.4$ mV; carbenoxolone, $29.7\pm1.3$ mV, $n=4$) was not modified in the presence of carbenoxolone (Figure 3a,c).

In the intact mesenteric artery the smooth muscle was also depolarized (by $5.6\pm1.0$ mV) by 100 $\mu$M carbenoxolone (Figure 3b,d). Under these conditions, the hyperpolarizations to 5 mM KCl (control, $16.8\pm0.4$ mV; carbenoxolone, $16.9\pm1.2$ mV, $n=4$) and to 10 $\mu$M levcromakalim (control, $26.8\pm0.6$ mV; carbenoxolone, $27.8\pm1.4$ mV, $n=4$) were not modified whereas hyperpolarization to 10 $\mu$M acetylcholine (control, $24.2\pm1.1$ mV; carbenoxolone, $18.3\pm1.3$ mV, $n=4$; $P<0.05$) was somewhat attenuated (Figure 3b,d).

In the presence of phenylephrine (1 $\mu$M), the smooth muscle cells of intact hepatic and mesenteric arteries were depolarized (by approximately 16 mV and 10 mV, respectively) relative to those in the absence of this agonist (compare Figures 3 and 5). Under these conditions, 100 $\mu$M carbenoxolone induced a further depolarization in both vessels (hepatic artery, $6.2\pm0.6$ mV, $n=4$: mesenteric artery, $3.9\pm1.4$ mV, $n=5$; Figure 4, 5). In contrast, 500 $\mu$M gap 27 had no effect on the membrane potential in the mesenteric artery but partially inhibited the depolarizing action of phenylephrine in the hepatic artery (depolarization approximately 11 mV). In the hepatic artery, acetylcholine-induced hyperpolarizations were inhibited both by carbenoxolone and by gap 27 (control, $26.0\pm0.5$ mV; carbenoxolone, $20.7\pm1.1$ mV, $n=4$, $P<0.05$; gap 27, $20.5\pm1.1$ mV, $n=4$, $P<0.05$), whereas only carbenoxolone reduced the effects of KCl (control hyperpolarization, $17.7\pm0.7$ mV; carbenoxolone, $14.9\pm1.2$ mV, $n=4$, $P<0.05$; gap 27, $16.4\pm1.1$ mV, $n=4$, $P>0.05$). In the mesenteric artery, hyperpolarizations to both acetylcholine ($27.1\pm0.7$ mV, $n=4$) and KCl ($19.4\pm0.8$ mV, $n=4$) were inhibited by carbenoxolone (acetylcholine-induced hyperpolarization, $15.3\pm2.6$ mV, $n=4$, $P<0.05$; KCl-induced hyperpolarization, $16.1\pm1.3$ mV, $n=4$, $P<0.05$) and by gap 27 (acetylcholine-induced hyperpolarization, $14.6\pm0.6$ mV, $n=4$, $P<0.05$; KCl-induced hyperpolarization, $11.5\pm0.8$ mV, $n=4$, $P<0.05$).

The inhibitory effect of carbenoxolone in rat hepatic and mesenteric arteries was small compared with its effect in the guinea-pig internal carotid artery. In order to determine whether this was due to different sensitivities of the gap junctions in the rat and guinea-pig vessels to carbenoxolone, the effects of a higher concentration of this agent (500 $\mu$M) were determined in the rat hepatic artery in the presence or absence of phenylephrine (Figure 6). This concentration of carbenoxolone depolarized the preparations (carbenoxolone alone, $17.3\pm1.3$ mV, $n=3$; phenylephrine + carbenoxolone, $10.5\pm1.6$ mV, $n=3$) to a greater extent than 100 $\mu$M (see above and Figures 5 and 6). In the presence, but not in the absence of phenylephrine, the magnitude of the hyperpolarization induced both by acetylcholine ($28.4\pm1.0$ mV, $n=3$) and by KCl ($20.3\pm0.7$ mV, $n=3$) was decreased in the presence of 500 $\mu$M carbenoxolone ($20.2\pm1.0$ mV, $n=3$ and $14.7\pm0.6$ mV, $n=3$, respectively; Figure 6).

The small inhibitory effect of gap 27 on the rat preparations following simple pre-incubation of the vessels (Figure 5) may have been due to rapid wash-out of the peptide. To exclude this, segments of rat hepatic artery were pre-incubated for 1 h with 500 $\mu$M gap 27 and then exposed to 10 $\mu$M acetylcholine in the continued presence of the gap junction inhibitor. Under these conditions, the peak hyperpolarization to acetylcholine was not different from that seen in the superfused vessels and gap 27 still had no effect on the response to acetylcholine (Figure 6c,d).

## Discussion

### *Effects of carbenoxolone and gap 27*

In the guinea-pig isolated internal carotid artery, carbenoxolone and gap 27 each inhibited acetylcholine-induced, endothelium-dependent hyperpolarizations. Furthermore, the hyperpolarizing effect of 1-EBIO, a selective opener of endothelial cell $K^+$ channels (which produces endothelium-dependent hyperpolarization of smooth muscle cells but which does not provoke direct hyperpolarization of vascular smooth muscle cells: Edwards *et al.*, 1999), was also significantly inhibited by the two gap junction inhibitors. These observations collectively provide powerful evidence that gap junctions play a critical role in transmitting endothelial cell hyperpolar-

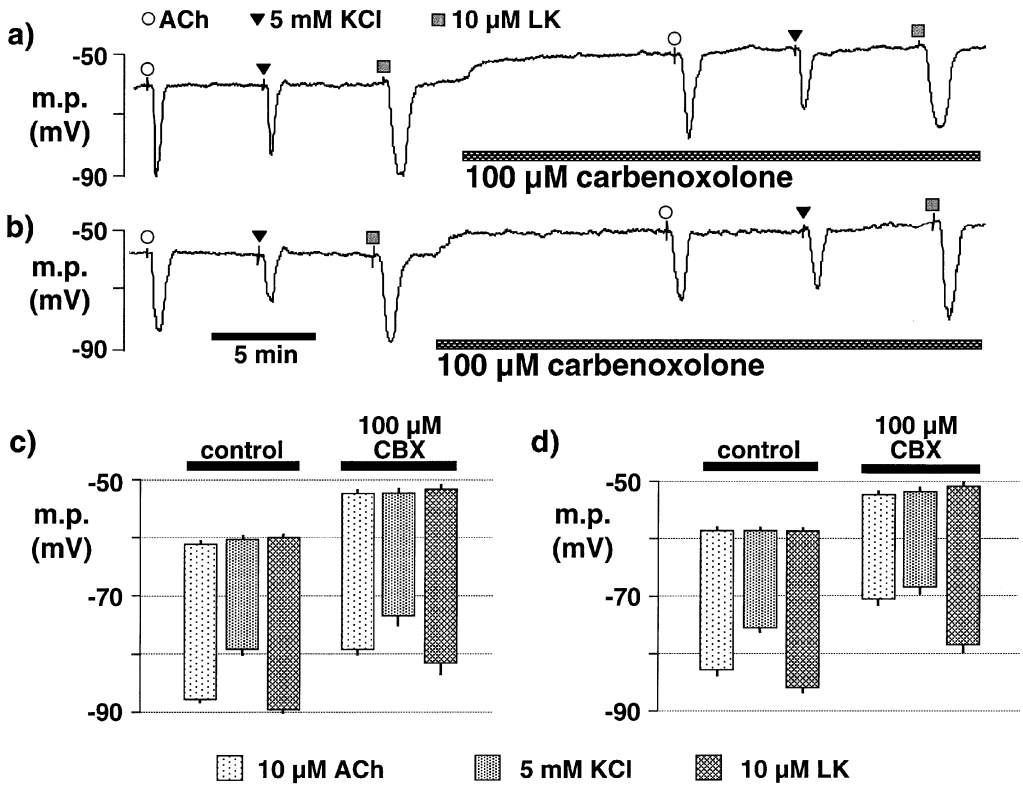


**Figure 3** Effects of carbenoxolone on hyperpolarizations induced by acetylcholine (ACh), potassium chloride (KCl) and levcromakalim (LK) in smooth muscle cells of rat intact hepatic (a,c) and mesenteric (b,d) arteries. (a,b) Acetylcholine, KCl and levcromakalim each hyperpolarized the smooth muscle cells of both vessels. Exposure to carbenoxolone depolarized the cells of both arteries but responses to acetylcholine, KCl and levcromakalim were essentially unchanged. (c,d) Data from four separate experiments are shown graphically. The columns represent the membrane potential (m.p.) ± s.e.mean before and after exposure to each agent in the absence or presence of carbenoxolone (CBX) as indicated.

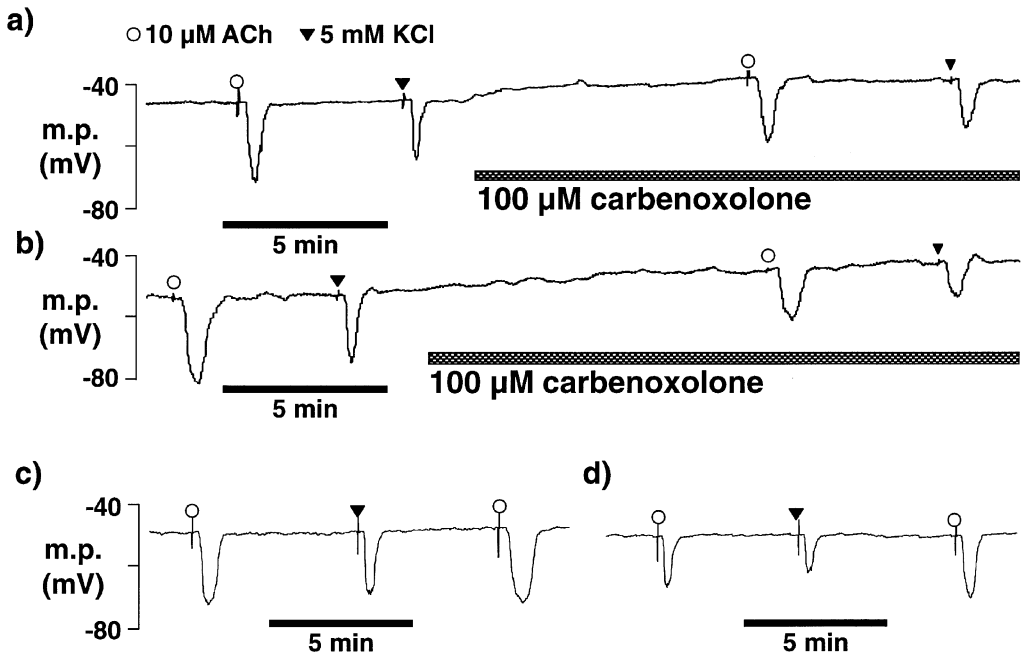


**Figure 4** Typical traces showing effects of 100 $\mu$M carbenoxolone (a,b) and 500 $\mu$M gap 27 (c,d) on responses to acetylcholine (ACh) and potassium chloride (KCl) in segments of rat hepatic (a,c) and rat mesenteric (b,d) arteries pretreated with 1 $\mu$M phenylephrine. (a,b) The muscle cells of both the hepatic and mesenteric arteries were approximately 10 mV depolarized by phenylephrine (compare with Figure 3) and both acetylcholine and KCl produced marked membrane potential (m.p.) hyperpolarizations. After exposure to carbenoxolone or in vessels pretreated with gap 27, the acetylcholine- and KCl-induced hyperpolarizations were slightly attenuated in the hepatic artery but more markedly reduced in the mesenteric artery. The horizontal bar beneath each trace represents 5 min.

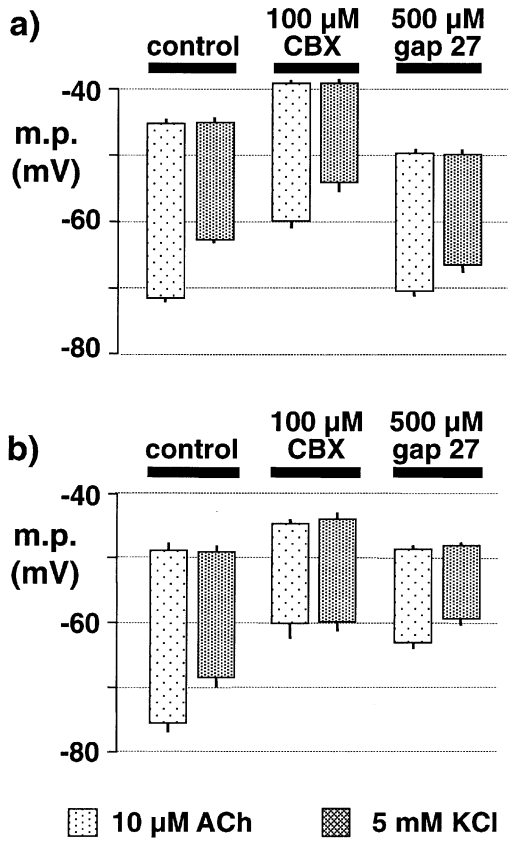


**Figure 5** Mean effects of carbenoxolone and gap 27 on responses to acetylcholine (ACh) and potassium chloride (KCl) in segments of rat hepatic (a) and rat mesenteric (b) arteries pre-treated with 1 $\mu$M phenylephrine. The columns represent the membrane potential (m.p.) $\pm$ s.e.mean, $n=4$, before and after exposure to each agent in the absence or presence of carbenoxolone (CBX) or gap 27 as indicated.

ization to the smooth muscle in the internal carotid artery of the guinea-pig. They are consistent with the observations made by Yamamoto *et al.* (1998; 1999) in small mesenteric arteries of the same species. However, whether the site of action of these inhibitors is on gap junctions between the endothelial and smooth muscle layers or solely within the smooth muscle remains to be established.

In the guinea-pig carotid artery and in the hepatic and mesenteric arteries of the rat, carbenoxolone also produced a depolarization, an effect which was endothelium-independent and not mimicked by gap 27. This was probably not the result of gap junction uncoupling but due rather to inhibition of a $Na^+/K^+$-ATPase, an ancillary property of carbenoxolone (Terasawa *et al.*, 1992). Carbenoxolone also depolarized guinea-pig carotid artery endothelial cells but it did not modify the response to acetylcholine, a further indication that its inhibitory action resulted from gap junction inhibition rather than a non-specific effect on the endothelium.

The effects of acetylcholine in unstimulated rat mesenteric artery (i.e. in the absence of phenylephrine) were significantly reduced by exposure to carbenoxolone but the magnitude of the inhibition was tiny compared with the effect seen in the guinea-pig carotid. Carbenoxolone was wholly without effect against acetylcholine-induced hyperpolarizations in the non-stimulated rat hepatic artery, even at a concentration of 500 $\mu$M. Collectively, therefore, gap junctions seem to play little or no role in transmitting endothelial cell hyperpolarizations to the smooth muscle cells in these vessels under 'basal' conditions. These findings are thus consistent with those of Edwards *et al.* (1998).

### *Gap junctions in stimulated vessels*

Although there was little evidence of a role for gap junctions in the EDHF response in the hepatic and mesenteric artery under 'basal' conditions, both carbenoxolone and gap 27 reduced endothelium-dependent hyperpolarizations when these vessels were stimulated using phenylephrine.

Within cells of the vasculature, gap junctions allow the transfer of small molecules and ions such as inositol trisphosphate and calcium (see for example, Carter *et al.*, 1996; Dora *et al.*, 1997). Thus the apparently increased role for gap junction coupling in the presence of phenylephrine (which increases smooth muscle intracellular calcium and inositol trisphosphate) may reflect the importance of these structures during contraction. If such processes modulate the open probability of calcium-sensitive $K^+$ channels in the endothelium, the observed 'EDHF' response on exposure to acetylcholine should indeed become more dependent on gap junctions in the presence of phenylephrine. Other factors such as the sensitivities of $Na^+/K^+$-ATPase isoforms to calcium and to phosphorylation by protein kinase C (Blanco & Mercer, 1997; Blanco *et al.*, 1998) could also play a role in the presence of a spasmogen.

In both the hepatic and the mesenteric arteries of the rat, $K^+$-induced hyperpolarizations were also somewhat inhibited by the gap junction inhibitors. Although inhibition of $Na^+/K^+$-ATPase (Terasawa *et al.*, 1992) could explain the action of carbenoxolone, the ability of gap 27 to reduce the effects of $K^+$ in the mesenteric (but not hepatic) artery is surprising and the subject of further study.

### *Consequences of gap junction inhibition*

The mechanism by which gap junctions couple endothelial cell hyperpolarization to the smooth muscle was beyond the scope of this study. However, it is possible that efflux of $K^+$ from endothelial cells (through $SK_{Ca}$ and/or $IK_{Ca}$) creates both electrical and chemical gradients which drive $K^+$ from the myocytes through the junctions to the endothelial cells, thus hyperpolarizing the smooth muscle. The elevation of extracellular $K^+$ would be tempered by the uptake of $K^+$ by $Na^+/K^+$-ATPase on each cell type, a process which could lead to further hyperpolarization of the endothelial cells (and potentially also of the smooth muscle in some tissues).

### *$K^+$-induced vascular hyperpolarization*

Hyperpolarization of the hepatic and mesenteric artery smooth muscle by a small increase in extracellular $K^+$ has been attributed to stimulation of inwardly-rectifying $K^+$ channels and to activation of $Na^+/K^+$-ATPase (Edwards *et al.*, 1998). In the rat hepatic artery, in the presence of 30 $\mu$M barium (to inhibit the inwardly-rectifying $K^+$ channels), the $K^+$-induced smooth muscle hyperpolarization is abolished by 500 nM ouabain (Edwards *et al.*, 1999).

Although the subtype of $Na^+/K^+$-ATPase responsible for the hyperpolarizing effect of $K^+$ has not been identified, its ouabain sensitivity in the rat hepatic artery (Edwards *et al.*, 1999) suggests that it is more likely to contain an $\alpha 2$ and/or $\alpha 3$ isoform rather than the less ouabain-sensitive, $\alpha 1$ subtype (see Blanco & Mercer, 1998). In addition, the affinities of the $\alpha 2$ and $\alpha 3$ isoforms for $K^+$ are lower than that of the $\alpha 1$ subtype, further supporting the possibility of an enhanced role for these

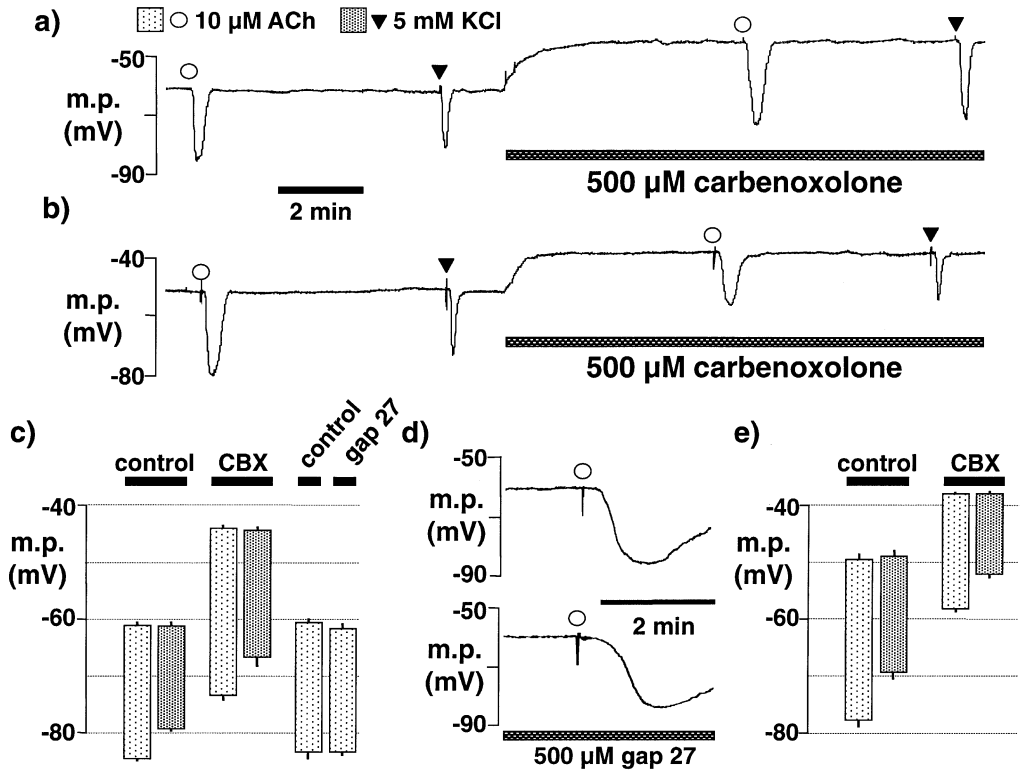


**Figure 6** Effects of 500 $\mu$M carbenoxolone and of the continuous presence of 500 $\mu$M gap 27 on hyperpolarizations produced by acetylcholine (ACh) and potassium chloride (KCl) in segments of rat hepatic artery. (a,b) typical traces showing the effects of carbenoxolone in the absence (a) and presence (b) of 1 $\mu$M phenylephrine. (c,e) data from three separate experiments of the type shown in (a) and (b), respectively, are shown graphically. The columns represent the membrane potential (m.p.) $\pm$ s.e.mean before and after exposure to each agent in the absence or presence of carbenoxolone (CBX) as indicated. (d) typical traces showing the hyperpolarization induced by acetylcholine in the absence (upper trace) and after 60 min pre-incubation and in the continuing presence of gap 27. The data from four separate experiments are shown in the two right-hand columns in (c).

forms when extracellular $K^+$ is elevated (see Blanco & Mercer, 1998). Thus, the lack of $K^+$-induced hyperpolarization in the guinea-pig carotid artery (present study; Quignard *et al*., 1999) may not only be due to poor expression of inwardly-rectifying $K^+$ channels (see Quignard *et al*., 1999) but also to the absence of $\alpha$2 and/or $\alpha$3 components from the $Na^+/K^+$-ATPase in this vessel. Characterization of these subunits in both the smooth muscle and endothelial cells of the guinea-pig internal carotid and rat mesenteric and hepatic arteries is currently in progress.

## *Conclusions*

Collectively, these data suggest that the initial stimulus for endothelium-related hyperpolarization of the vascular smooth muscle is the opening of calcium-sensitive $K^+$ channels on endothelial cell. In some preparations, such as the rat hepatic and mesenteric arteries, gap junctions play some role in the EDHF response. This phenomenon is nevertheless predominantly due to a direct stimulatory action of the $K^+$ (which exits from the endothelial cell $K^+$ channels) on the $Na^+/K^+$-ATPase and inwardly-rectifying $K^+$ channels of the surrounding smooth muscle cells (see Edwards *et al*., 1998). However, in other vessels such as the guinea-pig internal carotid artery, in which there is no direct effect of $K^+$ on the smooth muscle, the hyperpolarization may be propagated electrotonically from the endothelial cells *via* gap junctions. At present there are insufficient data to say whether these differences are species- and/or vessel-specific and further studies of the relative importance of gap junctions in the EDHF response in other vessels are underway.

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