ION CHANNELS – MEMBRANE TRANSPORT – INTEGRATIVE PHYSIOLOGY

Characterization of the endothelium-derived hyperpolarizing factor (EDHF) response in the human interlobar artery

ECKHART BÜSSEMAKER, RÜDIGER POPP, JOCHEN BINDER, RUDI BUSSE, and INGRID FLEMING

Institut für Kardiovaskuläre Physiologie, and Klinik für Urologie und Kinderurologie, Klinikum der J.W.G.-Universität, Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany

Characterization of the endothelium-derived hyperpolarizing factor (EDHF) response in the human interlobar artery.

Background. In addition to nitric oxide (NO) and prostacyclin (PGI_2), the vascular endothelium can influence local vascular tone by a mechanism involving the hyperpolarization of vascular smooth muscle cells. This response is attributed to the release of an endothelium-derived hyperpolarizing factor (EDHF). The present study was performed to determine the characteristics of the EDHF that mediates the NO/PGI_2-independent hyperpolarization and relaxation of human renal interlobar arteries.

Methods. Acetylcholine-induced, EDHF-mediated hyperpolarization and relaxation were assessed using sharp microelectrodes impaled into interlobar smooth muscle cells and in organ chamber experiments, respectively. All experiments were performed in the combined presence of NO synthase (NOS) and cyclooxygenase inhibitors and the thromboxane analog U46619.

Results. Interlobar arteries demonstrated pronounced NO/PGI_2-independent relaxations and hyperpolarizations that were sensitive to the blockade of calcium-activated K^+-channels (K_{Ca}^+ channels) by the combination of charybdotoxin and apamin and to the inhibition of the Na-K-ATPase by ouabain. Exogenously applied KCl also exhibited relaxations and hyperpolarizations that were sensitive to ouabain but insensitive to the combined inclusion of charybdotoxin and apamin. Relaxations induced by KCl were also observed in endothelium-denuded interlobar arteries.

Conclusion. These results indicate that in the human renal interlobar artery, EDHF-mediated responses display the pharmacologic characteristics of K^+ ions released through endothelial K_{Ca}^+ channels. Smooth muscle cell hyperpolarization and relaxation appear to be dependent on the activation of ouabain-sensitive subunits of the Na-K-ATPase.

In many vascular beds flow-dependent and agonist-induced vasodilatation are not regulated by either nitric oxide (NO) or prostacyclin (PGI_2) but by a mechanism

which involves the activation of Ca^{2+}-dependent K^+ (K_{Ca}^+) channels and is exquisitely sensitive to the combination of charybdotoxin and apamin. Although such responses were initially attributed to the release of an endothelium-derived hyperpolarizing factor (EDHF) from endothelial cells which activated K_{Ca}^+ channels on the underlying vascular smooth muscle, it is now accepted that EDHF-mediated responses are linked to the activation of endothelial K_{Ca}^+ channels and that the subsequent endothelial cell hyperpolarization is a prerequisite for the transfer of the signal to vascular smooth muscle cells, a decrease in muscle Ca^{2+} levels and relaxation [1].

A series of different mechanisms have been proposed to explain the EDHF phenomenon (for review see [2, 3]); however, there are currently three main pathways that could account for the majority of experimental effects observed. The first mechanism links endothelial cell stimulation with the activation of cytochrome P450 enzymes and the generation of hyperpolarizing metabolites of arachidonic acid [epoxyeicosatrienoic acids (EETs)], which either initiate the endothelial cell hyperpolarization or are released from endothelial cells to stimulate K_{Ca}^+ on vascular smooth muscle cells [4, 5]. The second mechanism involves the release of K^+ ions through activated endothelial K_{Ca}^+ channels, which then accumulate within the subintimal space in sufficient concentration to activate either inwardly rectifying K^+ channels or the Na-K-ATPase and thus elicit vascular smooth muscle hyperpolarization [6]. A third cellular mechanism, which could account for EDHF-mediated relaxation and which appears to play a significant role in resistance-sized arteries [7], involves the transfer of a hyperpolarizing signal [eventually via a cyclic adenosine monophosphate (cAMP)-dependent mechanism] from endothelial cells to smooth muscle cells through myo-endothelial gap junctions [8–11]. Hybrid arteries also exist in which EDHF-mediated relaxation is dependent on both a cytochrome P450 epoxygenase and gap junctional communication [12, 13].

EDHF-mediated responses have generally been char-

acterized using arteries isolated from experimental animals and relatively little information is available regarding the class of EDHF which predominates in human arteries [14–19]. The aim of the present investigation was to characterize the agonist-induced NO/PGI_2-independent relaxation of human renal interlobar arteries and to determine which EDHF mechanism mediates this response.

METHODS

Materials

All compounds were prepared as stock solutions in water, diluted in the experimental solution, and applied to the bath. Ouabain was dissolved in dimethyl sulphoxide (DMSO), so that the concentration did not exceed 0.1% in the final solution. Charybdotoxin was from Bachem (Heidelberg, Germany) and all other substances were from Sigma (Deisenhofen, Germany).

Vessel preparation

Human kidneys were obtained during nephrectomy as a result of renal carcinoma and interlobar arteries were excised from tissue that was considered to be free of carcinoma by the surgeon. Excision of the arteries was performed immediately after nephrectomy in the operating room under sterile conditions. Arteries were dissected, cleaned from adventitial adipose and connective tissue, and cut into rings (3 to 4 mm) for organ bath studies. The period between isolation and the start of experiments was less than 2 hours. Subjects were excluded if they had arterial hypertension (defined by blood pressure above 140/90 mm Hg measured on three different occasions), renal insufficiency (serum creatinine above 140 μmol/L), known cardiovascular disease (angina pectoris, previous myocardial infarction or coronary artery bypass grafting), diabetes (fasting serum glucose above 7 mmol/L), or if the nephrectomized kidney was greater than 9 cm in its longitudinal diameter measured by ultrasound or computed tomography. The protocol was approved by the local ethics committee. Interlobar arteries from 15 patients (54 ± 1.4 years; nine female and six male) were used. Due to the limited number of arteries, subgroup analysis with regard to differences in vascular reactivity due to gender or age was not performed.

Vascular reactivity studies

Renal interlobar arteries were mounted between force transducers (Hugo Sachs Elektronik-Harvard Apparatus, Germany) and a rigid support for measurement of isometric force and incubated in organ baths containing warmed (37°C), oxygenated (20% O_2, 5% CO_2 and 75% N_2 to give a pO_2 of approximately 140 mm Hg and pH 7.4 at 37°C) modified Tyrode's solution of the following composition: 132 mmol/L NaCl; 4 mmol/L KCl; 1.6 mmol/L $CaCl_2$; 0.98 mmol/L $MgCl_2$; 23 mmol/L $NaHCO_3$; 0.36 mmol/L NaH_2PO_4; 10 mmol/L glucose; 0.05 mmol/L Ca-Titriplex.

Passive tension was gradually adjusted over a 120-minute period to 2 g, thereafter segments were exposed to a modified Tyrode's solution rich in KCl (80 mmol/L) until stable contractions were obtained. After washing, arterial rings were exposed to the thromboxane analog U46619 (0.01 to 0.5 μmol/L) to achieve a stable contraction, which was approximately 80% of the maximal KCl-induced contraction. Thereafter, the integrity of the endothelium was assessed by the application of acetylcholine (1 μmol/L) and vessels exhibiting less than 80% relaxation were discarded. Experiments were performed in the continuous presence of diclofenac (10 μmol/L) and, except for the experiments evaluating NO responses, in the continuous presence of N^{ω}nitro-L-arginine (L-NA, 300 μmol/L). Agonist-induced relaxations were studied by performing cumulative concentration-response curves to acetylcholine (1 nmol/L to 1μmol/L). To determine the response to the cumulative application of KCl, the extracellular concentration of KCl was increased in steps of 1 mmol/L from 4 to 8 mmol/L.

Electrophysiologic measurements

Membrane potential in native vascular smooth muscle cells was measured as described [20]. Briefly, rings of interlobar artery (4 to 5 mm) were carefully slit along the longitudinal axis and pinned adventitial side downward to the bottom of a chamber and continuously superfused (1 mL/minute) with Tyrode's solution containing diclofenac (10 μmol/L), L-NA (300 μmol/L), and U46619 (100 nmol/L) to mimic conditions in the organ chamber. Smooth muscle membrane potentials were measured using conventional microelectrodes, pulled with a vertical pipette puller (HEKA Elektronik, Lambrecht/Pfalz, Germany) from filamented borosilicate glass tubing (Science Products, Hofheim, Germany). Microelectrodes were filled with 3 mol/L KCl. The electrical resistance of the electrodes, measured in modified Tyrode's solution, was 50 to 80 MΩ. Impalements were performed from the intimal side of the vessel. The potential measured was followed on an oscilloscope and traced with a pen recorder. Primary criteria for successful impalement were a sharp voltage deflection upon entering the cell, as judged visually on the oscilloscope, and a sharp return to the baseline value upon withdrawal of the pipette.

Statistics

Data are expressed as the mean ± SEM and statistical evaluation was performed using Student t test for paired or unpaired data, one-way analysis of variance (ANOVA) followed by a Bonferroni t test, or ANOVA for repeated measures where appropriate. Values of $P <$
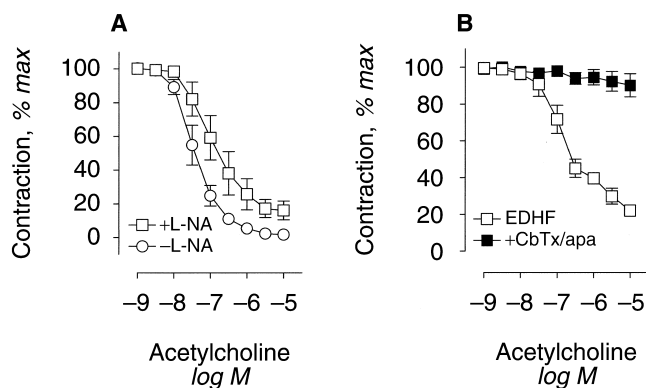
Fig. 1. Pharmacologic characterization of nitric oxide/prostacyclin (NO/PGI₂)-independent relaxation of the human interlobar artery. (*A*) Arterial rings were preconstricted with U46619 (0.01 to 0.5 μmol/L) in the continuous presence of diclofenac (10 μmol/L) and concentration-relaxation curves to acetylcholine (0.1 nmol/L to 10 μmol/L) were obtained in the absence (−L-NA) and presence of N$^\omega$-nitro-L-arginine (+L-NA, 300 μmol/L). (*B*) Arterial rings were preconstricted with U46619 in the continuous presence of diclofenac and L-NA and concentration-relaxation curves to acetylcholine were obtained in the absence of EDHF and presence of the combination of charybdotoxin (CbTx) (100 nmol/L) and apamin (apa) (100 nmol/L). Results are presented as the mean ± SEM of five separate experiments.

Fig. 2. Effect of inhibiting cytochrome P450 and the Na-K-ATPase on endothelium-derived hyperpolarizing factor (EDHF)-mediated relaxation. Concentration-relaxation curves to acetylcholine (0.1 nmol/L to 10 μmol/L) were obtained in rings preconstricted with U46619 (0.01 μmol/L to 0.5 μmol/L) in the absence (EDHF) and presence of (*A*) sulfaphenazole (10 μmol/L) or (*B*) ouabain (100 nmol/L). All experiments were performed in the continuous presence of diclofenac (10 μmol/L) and L-NA (300 μmol/L) and the results are presented as the mean ± SEM of five separate experiments.

0.05 were considered statistically significant. R_{max} represents the maximal relaxation recorded in response to the cumulative addition of a given agonist and pD_2 (–log EC_{50}) values were calculated by nonlinear regression of the concentration-relaxation curves.
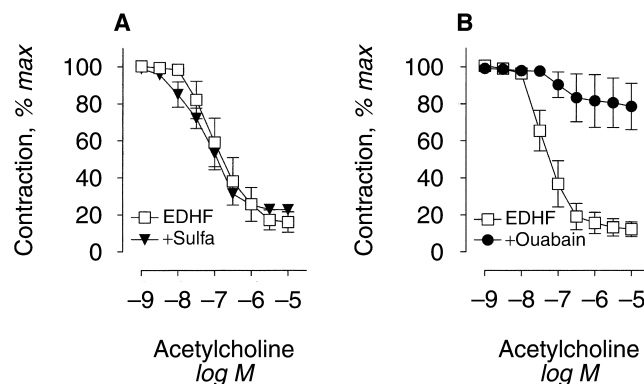
RESULTS

NO/PGI₂-independent relaxation

In U46619-precontracted rings of renal interlobar artery, acetylcholine elicited concentration-dependent relaxations. The inclusion of L-NA (300 μmol/L) in the organ bath slightly attenuated maximal acetylcholine-induced relaxation (from 98.0 ± 0.7% to 84.2 ± 5.5%, $N = 6$, $P < 0.05$) and resulted in a significant rightward shift in the concentration-relaxation curve to acetylcholine (pD_2 values were 7.5 ± 0.1 versus 6.9 ± 0.2 in the absence and presence of L-NA, $N = 6$, $P < 0.05$; Fig. 1A).

The combination of charybdotoxin (100 nmol/L), a blocker of intermediate and large conductance K_{Ca}^+ channels and apamin (100 nmol/L), a blocker of small-conductance K_{Ca}^+ channels, almost completely prevented acetylcholine-induced, NO/PGI₂-independent relaxation (Fig. 1B). Since the sensitivity to charybdotoxin/apamin is a hallmark of EDHF-mediated responses, the NO/PGI₂-independent relaxation of the interlobar artery is subsequently referred to as an EDHF-mediated relaxation.

Characterization of the EDHF-mediated relaxation

Sulfaphenazole (10 μmol/L), a specific inhibitor of CYP 2C9, which inhibits the bradykinin-induced, EDHF-

mediated relaxation of large porcine coronary arteries [5], did not affect the concentration-relaxation curve to acetylcholine in human interlobar arteries (Fig. 2A).

As blockade of the Na-K-ATPase by ouabain has been reported to attenuate EDHF-mediated hyperpolarization of some human subcutaneous arteries [18, 21], we determined the effects of this inhibitor on the acetylcholine-induced relaxation of rings of human interlobar arteries. The concentration of ouabain used (100 nmol/L) did not affect basal tension (data not shown) but attenuated the acetylcholine-induced EDHF-mediated relaxation. R_{max} was reduced from 91.9 ± 5.0% to 19.7 ± 10.5% ($P < 0.01$, $N = 5$; Fig. 2B).

### Comparison of EDHF-mediated and KCl-induced relaxation

Low concentrations of KCl (4 to 10 mmol/L) are reported to activate the Na-K-ATPase in vascular smooth muscle cells [22], and increasing the extracellular concentration of KCl in steps of 1 mmol/L from 4 to 8 mmol/L elicited an endothelium-independent relaxation of interlobar artery rings (Fig. 3A). Equivalent concentrations of NaCl were without effect (data not shown). The KCl-induced relaxation was unaffected by the combination of charybdotoxin and apamin (each 100 nmol/L; Fig. 3B) but was abolished in vessels pretreated with ouabain (100 nmol/L, Fig. 3C).

Electrophysiologic measurements

Electrophysiologic experiments to record changes in smooth muscle membrane potential were performed in the presence of L-NA, diclofenac, and U46619 (100 nmol/L) to mimic the conditions under which tone was
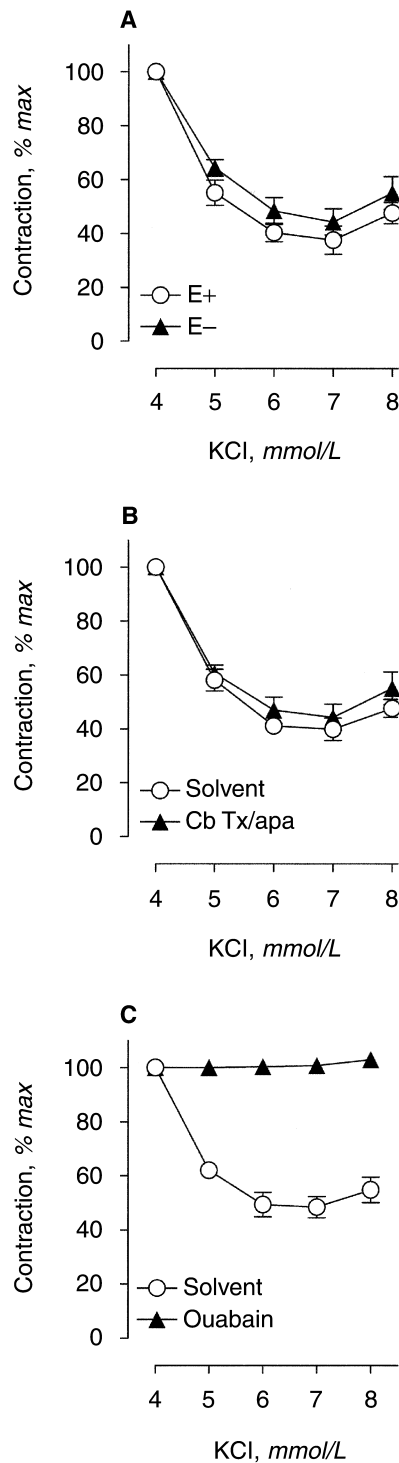
Fig. 3. **KCl-induced relaxation of the human interlobar artery.** Concentration-relaxation curves to KCl (4 to 8 mmol/L) were obtained in endothelium-intact (E+) and endothelium-denuded (E−) rings of human interlobar artery (*A*), in endothelium-intact arteries in the absence (Solvent) and presence of charybdotoxin and apamin (CbTx/apa, each 100 nmol/L) (*B*), or ouabain (100 nmol/L) (*C*). Rings were preconstricted with U46619 (0.01 μmol/L to 0.5 μmol/L). All experiments were performed in the continuous presence of diclofenac (10 μmol/L) and L-NA (300 μmol/L) and the results are presented as the mean ± SEM of five separate experiments.
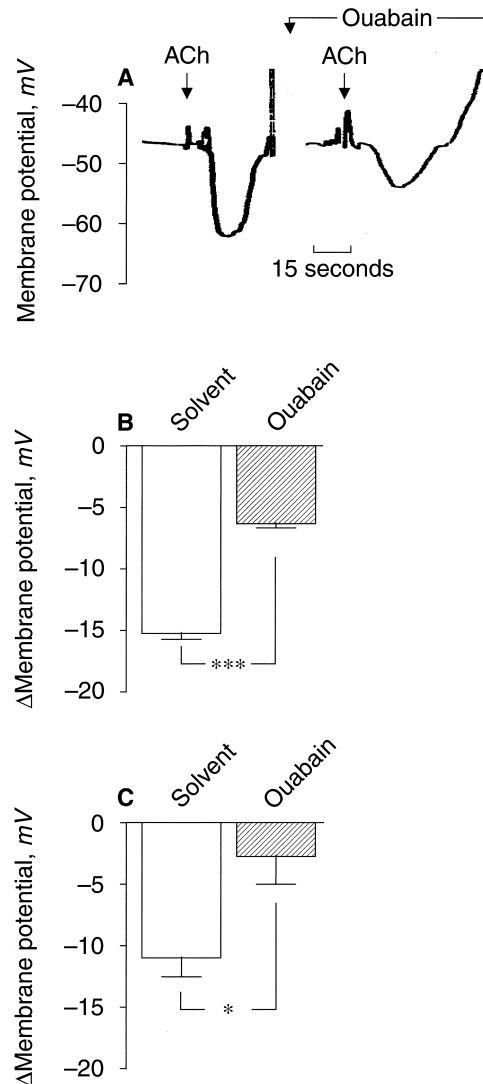
Fig. 4. **Effect of ouabain on the endothelium-derived hyperpolarizing factor (EDHF)- and KCl-induced hyperpolarization of the human interlobar artery.** (*A*) Original tracings showing the effect of acetylcholine (ACh, 1 μmol/L) on the membrane potential of human interlobar smooth muscle cells in the absence and presence of ouabain (100 nmol/L). (*B* and *C*) Bar graphs summarizing the effect of ouabain (100 nmol/L) on the change in membrane potential (mV) in interlobar smooth muscle cells following stimulation of the vessels with either (B) acetylcholine (1 μmol/L) or (C) KCl (final bath concentration 10 mmol/L). All experiments were performed in the continuous presence of diclofenac (10 μmol/L), L-NA (300 μmol/L), and U46619 (100 nmol/L) and the results are presented as the mean ± SEM of four separate experiments. *$P <$ 0.05; ***$P <$ 0.001.

assessed. Acetylcholine (1 μmol/L) elicited a significant, endothelium-dependent hyperpolarization of vascular smooth muscle cells (Fig. 4A). Ouabain (100 nmol/L) did not significantly depolarize U46619-precontracted arteries (the resting membrane potential being −46.8 ± 1.3 mV in the absence versus –42.5 ± 1.2 mV in the presence of ouabain, $N =$ 4 to 6) but markedly attenuated the acetylcholine-induced hyperpolarization (Fig. 4A).

Increasing the extracellular KCl concentration from 4 to 10 mmol/L resulted in the hyperpolarization of smooth muscle cells, which was also significantly inhibited in the presence of ouabain (Fig. 4B).

DISCUSSION

The results of the present study demonstrate that a substantial portion of the acetylcholine-induced relaxation of the human interlobar artery is mediated by a mechanism that does not involve the generation of either NO or PGI_2. This NO/PGI_2-independent relaxation was associated with the activation of the Na-K-ATPase and the hyperpolarization of smooth muscle cells.

NO/PGI_2-independent relaxations, which are associated with the hyperpolarization of vascular smooth muscle cells, are generally attributed to the generation and release of an EDHF. Although several mechanisms may be involved in the endothelium-dependent hyperpolarization and relaxation of smooth muscle cells, there is one characteristic that is shared by all of the EDHFs described to date and that is the absolute prerequisite for the activation of K_{Ca}^+ channels and the hyperpolarization of endothelial cells prior to the transfer of this signal to vascular smooth muscle cells. This dependence is highlighted by the sensitivity of the response to the combination of charybdotoxin and apamin [23]. As apamin inhibits small conductance K_{Ca}^+ channels, whereas charybdotoxin inhibits large and intermediate conductance K_{Ca}^+ channels, the toxin combination targets the whole range of K_{Ca}^+ channels expressed on the endothelium [24]. Endothelial cell hyperpolarization can therefore be initiated by hemodynamic stimuli as well as by agonists that increase endothelial $[Ca^{2+}]_i$ and activate K_{Ca}^+ channels or which elicit the generation of factors (e.g., EETs), which enhance the sensitivity of K_{Ca}^+ channels to Ca^{2+} [25]. The hyperpolarization of some arteries can be significantly attenuated by charybdotoxin [26] or iberiotoxin alone [27, 28] and this phenomenon is currently thought to reflect differences in the expression of K_{Ca}^+ channel subtypes on the endothelial cells in question.

Once the endothelial cell hyperpolarization has been transferred to the vascular smooth muscle, $[Ca^{2+}]_i$ is decreased partially due to the inhibition of Ca^{2+} entry through voltage-dependent Ca^{2+} channels [29]. Experimental evidence suggests that the hyperpolarization signal can be transferred from one cell type to the other by one (or more) of three mechanisms (i.e., by the release of a diffusible factor such as an EET, the release of K^+ ions from endothelial cells, and via activation of gap junctional communication).

Studies in humans [16] or using isolated human vessels [14, 17, 18] have described a NO/PGI_2-independent vasodilatation/relaxation that can be attenuated by cytochrome P450 inhibitors. This type of EDHF response is particularly prominent in arteries demonstrating endothelial dysfunction and an impaired NO-mediated vasodilator response to acetylcholine and increased flow, which has led to the suggestion that EDHF may act as a back-up vasodilator system when NO production or bioavailability is impaired [30, 31]. In the human interlobar artery, we found no evidence of the expression of cytochrome P450 2C (the enzyme identified as a putative EDHF synthase in porcine coronary arteries [5], data not shown) and no evidence to suggest that the acetylcholine-induced EDHF mediated hyperpolarization or relaxation of vascular smooth muscle is related to the activation of cytochrome P450 enzymes. This finding contrasts somewhat with the porcine interlobar artery since, although cytochrome P450 activity cannot account for the majority of the EDHF response in this artery, sulfaphenazole did induce a rightward shift in the EDHF-mediated concentration-relaxation response curve to bradykinin [32]. The transfer of endothelial cell hyperpolarization to smooth muscle cells via myo-endothelial gap junctions is an additional possibility, although this mechanism is more evident in small, resistance-sized arteries. However, due to the limited amount of human material available, it was not possible to thoroughly address the role of gap junctions in the EDHF-mediated relaxation of the human interlobar artery.

An increase in the concentration of K^+ ions in the intimal space between the endothelial and smooth muscle cell layers would be expected to activate inwardly rectifying K^+ channels and/or the Na-K-ATPase in smooth muscle cells and thus elicit hyperpolarization. There is evidence to suggest that a K^+-like EDHF may be physiologically relevant in humans since barium, which inhibits inwardly rectifying K^+ channels, is reported to reduce resting blood flow and inhibit potassium-induced vasodilatation in the human forearm [19] and, in patients with essential hypertension, ouabain attenuates the bradykinin-induced, NO/PGI_2-independent vasodilatation in the forearm vasculature [15]. However, we found no effect of barium (30 µmol/L) on the acetylcholine-induced EDHF-mediated relaxation of human interlobar arteries (authors unpublished observations), a finding that is consistent with reports that inwardly rectifying K^+ channels are expressed mainly in smaller resistance-sized arteries (<200 µm) (for review see [33]). In rabbit renal arcuate arteries, KCl-induced vasorelaxation is also reported to be sensitive to ouabain but insensitive to barium since inwardly rectifying K^+ channels have a low average density in arcuate artery smooth muscle cells [34].

In the human interlobar artery, the EDHF-mediated relaxation was abolished by the combination of charybdotoxin and apamin and could be mimicked by increasing the extracellular concentration of KCl. Both the EDHF- and the KCl-induced hyperpolarization and relaxation of human interlobar arteries were sensitive

to a low concentration of ouabain, suggesting that the EDHF-mediated hyperpolarization of the human interlobar artery involves the activation of K_{Ca}^+ channels, the release of K^+ from endothelial cells, and the subsequent activation of the Na-K-ATPase. The hypothesis that the efflux of K^+ ions through endothelial charybdotoxin and apamin-sensitive K_{Ca}^+ channels can account for the EDHF phenomenon is attractive but highly controversial and, given the attenuated sensitivity of precontracted vessels to an increase in extracellular KCl [35], is unlikely to contribute to EDHF-mediated responses in all arteries that demonstrate an agonist-induced, NO/PGI$_2$-independent relaxation. The interlobar artery, however, is sensitive to modest increases in the extracellular KCl concentration, even when contracted suggesting that small variations in extracellular KCl could modulate blood flow in the renal circulation. This could have important implications for the regulation of vascular tone in pathologic conditions that are accompanied by a decrease in the bioavailability of NO since EDHF could act as a compensatory vasodilator mechanism. Indeed, as mentioned above, in patients with essential hypertension endothelium-dependent vasodilatation is impaired because of reduced NO availability and in hypertensive subjects, bradykinin-induced vasodilatation is not modified by an NOS inhibitor but is reported to be significantly reduced by ouabain [15]. Hence, the mechanism described here in the interlobar artery has physiologic and pathophysiologic importance for the regulation of vascular tone. Although the interlobar artery is not a typical resistance-sized artery and the impact of its regulation on renal vascular resistance is likely to be rather limited, a similar mechanism in smaller arteries would be expected to significantly affect renal blood flow.

ACKNOWLEDGMENTS

Reprint requests to Ingrid Fleming, Institut für Kardiovaskuläre Physiologie, Klinikum der J.W.G.-Universität, Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany.
E-mail: fleming@em.uni-frankfurt.de

REFERENCES

1. BUSSE R, EDWARDS G, FELETOU M, et al: EDHF: Bringing the concepts together. *Trends Pharmacol Sci* 23:374–380, 2002
2. FLEMING I, BUSSE R: EDHF: Update on an alternative vasodilator with potential renal significance. *Nephrol Dial Transplant* 13:2721–2723, 1998
3. MCGUIRE JJ, DING H, TRIGGLE CR: Endothelium-derived relaxing factors: A focus on endothelium-derived hyperpolarizing factor(s). *Can J Physiol Pharmacol* 79:443–470, 2001
4. CAMPBELL WB, GEBREMEDHIN D, PRATT PF, et al: Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. *Circ Res* 78:415–423, 1996
5. FISSLTHALER B, POPP R, KISS L, et al: Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature* 401:493–497, 1999
6. EDWARDS G, DORA KA, GARDENER MJ, et al: K$^+$ is an endothelium-derived hyperpolarizing factor in rat arteries. *Nature* 396:269–272, 1998
7. SHIMOKAWA H, YASUAAKE H, FUJII K, et al: The importance of the hyperpolarizing mechanism increases as the vessel size decreases in endothelium-dependent relaxations in rat mesenteric circulation. *J Cardiovasc Pharmacol* 28:703–711, 1996
8. DORA KA, MARTIN PE, CHAYTOR AT, et al: Role of heterocellular Gap junctional communication in endothelium-dependent smooth muscle hyperpolarization: inhibition by a connexin-mimetic peptide. *Biochem Biophys Res Commun* 254:27–31, 1999
9. GRIFFITH TM, CHAYTOR AT, TAYLOR HJ, et al: cAMP facilitates EDHF-type relaxations in conduit arteries by enhancing electrotonic conduction via gap junctions. *Proc Natl Acad Sci USA* 99:6392–6397, 2002
10. URAKAMI-HARASAWA L, SHIMOKAWA H, NAKASHIMA M, et al: Importance of endothelium-derived hyperpolarizing factor in human arteries. *J Clin Invest* 100:2793–2799, 1997
11. DE WIT C, ROOS F, BOLZ SS, et al: Impaired conduction of vasodilatation along arterioles in connexin40-deficient mice. *Circ Res* 86:649–655, 2000
12. BOLZ SS, FISSLTHALER B, PIEPERHOFF S, et al: Antisense oligonucleotides against cytochrome P450 2C8 attenuate EDHF-mediated Ca^{2+} changes and dilation in isolated resistance arteries. *FASEB J* 14:255–260, 2000
13. HOEPFL B, RODENWALDT B, POHL U, et al: EDHF, but not NO or prostaglandins, is critical to evoke a conducted dilation upon ACh in hamster arterioles. *Am J Physiol Heart Circ Physiol* 283:H996–H1004, 2002
14. MIURA H, GUTTERMAN DD: Human coronary arteriolar dilation to arachidonic acid depends on cytochrome P-450 monooxygenase and Ca^{2+}-activated K$^+$ channels. *Circ Res* 83:501–507, 1998
15. TADDEI S, GHIADONI L, VIRDIS A, et al: Vasodilation to bradykinin is mediated by an ouabain-sensitive pathway as a compensatory mechanism for impaired nitric oxide availability in essential hypertensive patients. *Circulation* 100:1400–1405, 1999
16. HALCOX JP, NARAYANAN S, CRAMER-JOYCE L, et al: Characterization of endothelium-derived hyperpolarizing factor in the human forearm microcirculation. *Am J Physiol Heart Circ Physiol* 208:H2470–H2477, 2001
17. MCINTYRE CA, BUCKLEY CH, JONES GC, et al: Endothelium-derived hyperpolarizing factor and potassium use different mechanisms to induce relaxation of human subcutaneous resistance arteries. *Br J Pharmacol* 133:902–908, 2001
18. COATS P, JOHNSTON F, MACDONALD J, et al: Endothelium-derived hyperpolarizing factor: Identification and mechanisms of action in human subcutaneous resistance arteries. *Circulation* 103:1702–1708, 2001
19. DAWES M, SIENIAWSKA C, DELVES T, et al: Barium reduces resting blood flow and inhibits potassium-induced vasodilation in the human forearm. *Circulation* 105:1323–1328, 2002
20. FISSLTHALER B, HINSCH N, CHATAIGNEAU T, et al: Nifedipine increases cytochrome P4502C expression and EDHF-mediated responses in coronary arteries. *Hypertension* 36:270–275, 2000
21. COLEMAN HA, TARE M, PARKINGTON HC: K$^+$ currents underlying the action of endothelium-derived hyperpolarizing factor in guinea-pig, rat and human blood vessels. *J Physiol* 531:359–373, 2001
22. WEBB RC, BOHR DF: Potassium-induced relaxation as an indicator of Na$^+$-K$^+$ ATPase activity in vascular smooth muscle. *Blood Vessels* 15:198–207, 1978
23. ZYGMUNT PM, HÖGESTÄTT ED: Role of potassium channels in endothelium-dependent relaxation resistant to nitroarginine in the rat hepatic artery. *Br J Pharmacol* 117:1600–1606, 1996
24. BARON A, FRIEDEN M, CHABAUD F, et al: Ca^{2+}-dependent nonselective cation and potassium channels activated by bradykinin in pig coronary artery endothelial cells. *J Physiol* 493:691–706, 1996
25. LI PL, CAMPBELL WB: Epoxyeicosatrienoic acids activate K$^+$ channels in coronary smooth muscle through a guanine nucleotide binding protein. *Circ Res* 80:877–884, 1997
26. DE WIT C, ESSER N, LEHR H-A, et al: Pentobarbital-sensitive EDHF comediates ACh-induced arteriolar dilation in the hamster microcirculation. *Am J Physiol* 276:H1527–H1534, 1999
27. NISHIKAWA Y, STEPP DW, CHILIAN WM: In vivo location and mech-

anism of EDHF-mediated vasodilation in canine coronary micro-circulation. *Am J Physiol* 277:H1252–H1259, 1999

28. HUANG A, SUN D, SMITH CJ, *et al*: In eNOS knockout mice skeletal muscle arteriolar dilation to acetylcholine is mediated by EDHF. *Am J Physiol Heart Circ Physiol* 278:H762–H768, 2000
29. BOLZ SS, DE WIT C, POHL U: Endothelium-derived hyperpolarizing factor but not NO reduces smooth muscle Ca^{2+} during acetylcholine-induced dilation of microvessels. *Br J Pharmacol* 128:124–134, 1999
30. BAUERSACHS J, POPP R, HECKER M, *et al*: Nitric oxide attenuates the release of endothelium-derived hyperpolarizing factor. *Circulation* 94:3341–3347, 1996
31. KIM H, SHIM J, HAN PL, *et al*: Nitric oxide modulates the c-Jun N-terminal kinase stress- activated protein kinase activity through

activating c-Jun N-terminal kinase kinase. *Biochemistry* 36:13677–13681, 1997
32. BÜSSEMAKER E, WALLNER C, FISSLTHALER B, *et al*: The Na-K-ATPase is a target for an EDHF displaying characteristics similar to potassium ions in the porcine renal interlobar artery. *Br J Pharmacol* 137:647–654, 2002
33. NELSON MT, QUAYLE JM: Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol* 268: C799–C822, 1995
34. PRIOR HM, WEBSTER N, QUINN K, *et al*: K^+-induced dilation of a small renal artery: No role for inward rectifier K^+ channels. *Cardiovasc Res* 37:780–790, 1998
35. DORA KA, INGS NT, GARLAND CJ: K_{Ca} channel blockers reveal hyperpolarization and relaxation to K^+ in rat isolated mesenteric artery. *Am J Physiol Heart Circ Physiol* 283:H606–H614, 2002