Kidney International, Vol. 61 (2002), pp. 177–185

Effects of connexin-mimetic peptides on nitric oxide synthase- and cyclooxygenase-independent renal vasodilation

An S. De Vriese, Johan Van de Voorde, and Norbert H. Lameire

Renal Unit and the Department of Physiology, University Hospital, Gent, Belgium

Effects of connexin-mimetic peptides on nitric oxide synthase- and cyclooxygenase-independent renal vasodilation.

Background. Research on the physiological role of endothelium-derived hyperpolarizing factor (EDHF) is hampered by the persistent controversy on its nature and mechanisms of action, as well as by the lack of specific inhibitors that are suitable for in vivo use. Recent in vitro studies support a role for gap junctions in EDHF-mediated signal transmission. The present study examines the contribution of gap junctional communication to the EDHF-mediated responses in the rat renal microcirculation in vivo and addresses the physiological role of EDHF.

Methods. The effects of intrarenal administration of connexin-mimetic peptides on the L-NAME- and indomethacin-resistant renal blood flow (RBF) response to acetylcholine, on basal RBF and on systemic blood pressure were examined.

Results. ^{43}Gap 27, a peptide homologous to the second extracellular loop of connexin 43, partially inhibited the L-NAME- and indomethacin-resistant RBF response to acetylcholine, whereas ^{40}Gap 27, homologous to the second extracellular loop of connexin 40, abolished the response. A control peptide, with a replacement of two amino acids in the motif SRPTEK present in the second extracellular loop of connexins 40 and 43, was without effect. None of the peptides affected the response to DETA-NONOate, pinacidil or papaverine. Intrarenal infusion of ^{43}Gap 27 or ^{40}Gap 27 decreased basal RBF and increased mean arterial blood pressure, both in the presence and absence of systemic infusion of L-NAME and indomethacin.

Conclusions. Inhibition of gap junctional communication with connexin-mimetic peptides blocks EDHF-mediated signal transmission in vivo, as suggested by the abolishment of L-NAME- and indomethacin-resistant renal vasodilation. The peptides also decrease basal RBF and increase blood pressure, supporting a role for tonic EDHF release in the control of tissue perfusion and vascular resistance.

The vascular endothelium synthesizes and releases vasoactive autacoids that regulate vascular smooth muscle tone and reactivity. Nitric oxide (NO) and prostacyclin are the best characterized vasodilator factors produced by the endothelium. Over the past two decades, several lines of evidence have convincingly demonstrated the existence of a third endothelium-derived vasodilator pathway, which is especially prominent in resistance arteries [1, 2]. Since the NO synthase- and cyclooxygenase-independent endothelium-mediated vasodilation is associated with vascular smooth muscle hyperpolarization, the term endothelium-derived hyperpolarizing factor (EDHF) was proposed [3]. The nature of EDHF is one of the most contentious questions in vascular physiology [4]. Current evidence suggests that EDHF is more than one substance and that the identity, as well as the mechanisms of action of EDHF show substantial tissue and species heterogeneity. Several candidate mediators have been proposed, including epoxyeicosatrienoic acids (EETs), which are cytochrome P450 mono-oxygenase derived metabolites of arachidonic acid [5–7], the endogenous cannabinoid anandamide [8], and potassium ions [9]. In some vessels, however, neither a cytochrome P450 metabolite nor a cannabinoid or potassium meets the pharmacological criteria for an EDHF. Except for the involvement of opening of K^+-channels on endothelial and/or smooth muscle cells, the mechanisms of the EDHF-pathway are still a matter of debate.

Although both cascade bioassay [10] and sandwich preparations [11] have suggested that EDHF can diffuse freely in the extracellular space, several lines of evidence support the direct transfer of EDHF through myoendothelial gap junctions. Functional dye transfer experiments demonstrate direct coupling between endothelial cells and the underlying smooth muscle cells [12]. Hyperpolarization of the smooth muscle cells is associated with hyperpolarization of the endothelial cells, supporting electrical coupling between the two cell types. Recently, myoendothelial gap junction plaques were demonstrated anatomically and appeared substantially more common in the distal than in the proximal regions of the vasculature [13]. This finding is commensurate with the well-known observation that the contribution of EDHF to endothelium-dependent vasodilatation increases as ves-

Key words: connexins, EDHF, gap junctions, renal microcirculation, blood flow, hypertension, signal transmission.

Received for publication April 23, 2001
and in revised form August 16, 2001
Accepted for publication August 31, 2001

sel size becomes smaller [14–18]. Finally, gap junction blockers inhibit NO synthase- and cyclooxygenase-independent relaxation in different artery types in vitro [19–25].

Gap junctions are formed when two connexons contributed by neighboring cells dock through interactions between their extracellular loops. A connexon consists of six connexins arranged around an aqueous central pore, that allows the transfer of electrical current and small molecules <1 kD in size. Multiple gap junctions, in turn, cluster to form gap junction plaques [26]. Despite the large number of connexins identified, only connexin 43, 40 and 37 are expressed in mammalian vasculatures [27]. Several pharmacological agents interfere with gap junction formation, including heptanol, the 18α and 18β isoforms of glycyrrhetinic acid and carbenoxolone, but these molecules may have unwanted aspecific effects when used in vivo. The use of connexin knockout mice [28] or of connexin-mimetic peptides [20–25] may be more specific means to interfere with gap junction function. Connexin-mimetic peptides possess sequence homology to specific regions of connexin proteins. The interactions between the extracellular loops of connexins are not fixed and immobile, but involve a dynamic docking-undocking mechanism. The connexin-mimetic peptides bind to the essential components of the connexin docking sites and thus interfere with the docking of two connexons contributed by neighboring cells.

Very little research concerning EDHF has been conducted in vivo, reflecting the toxicity of potassium channel blocking agents and the inability to directly measure endothelium-dependent hyperpolarization, as the hallmark of EDHF activity. Nevertheless, in vivo measurement of the contribution of EDHF to endothelium-dependent vasodilation may be important, as the magnitude of the response is determined by the resting membrane potential, which is quite different in arteries under physiological pressure in vivo than in those suspended in vitro [29]. Furthermore, in vivo research permits the investigation of the physiological relevance of EDHF, which is currently unknown.

The aim of the present study was to characterize the NO synthase- and cyclooxygenase-independent endothelium-mediated vasodilation in response to acetylcholine in the renal microcirculation in vivo. In particular, the potential contribution of gap junctional communication to the EDHF-response was evaluated by the use of connexin-mimetic peptides. In addition, the physiological role of EDHF was addressed by evaluating the effects of blockade of EDHF-mediated responses on basal renal blood flow and on systemic blood pressure.

METHODS

The studies were performed in 38 female Wistar rats with a body weight of ±250 g (Iffa Credo, Brussels, Belgium), receiving care in accordance with NIH and national guidelines for animal protection. The rats were anesthetized with thiobutabarbital (100 mg/kg i.p.; Inactin; RBI, Natick, MA, USA). The trachea was intubated, a jugular vein was cannulated for continuous infusion of isotonic saline (3 mL/h) and administration of drugs, and a carotid artery was cannulated for continuous monitoring of arterial blood pressure and recording of heart rate. The right renal and suprarenal arteries were exposed via a small abdominal incision. The suprarenal artery was cannulated for intrarenal administration of drugs. A blood flow sensor with an inner diameter of 0.6 to 0.8 mm was placed on the renal artery, allowing continuous renal blood flow monitoring by an electromagnetic square wave flow meter (Skalar Medical, Delft, The Netherlands) [18].

Experimental protocols

Series 1. All studies were performed in the continuous and combined presence of systemic NO synthase and cyclooxygenase blockade: L-N^G-nitroarginine methylester HCl (L-NAME; 10 mg/kg bolus followed by 20 mg/kg/h; Sigma Chemical Co, St. Louis, MO, USA) and indomethacin (4 mg/kg bolus followed by 8 mg/kg/h; Sigma).

The renal blood flow response to intrarenal acetylcholine (1 to 50 ng in bolus; Sigma), to the NO donor detaNONOate (16 to 80 μg in bolus; Alexis, Grünberg, Germany), to the K^+-channel opener pinacidil (25 to 125 μg in bolus; Sigma) and to papaverine (10 to 50 μg in bolus; Federa, Brussels, Belgium) was examined before and 5 and 30 minutes after infusion of ^{43}Gap 27 peptide (3.91 mg, sequence SRPTEKTIFII; synthesized by Sigma-Genosys (Cambridge, UK); $N = 8$), ^{40}Gap 27 peptide (3.87 mg, sequence SRPTEKNVFIV; $N = 6$), a control peptide (3.62 mg, sequence SRGGEKNVFIV; $N = 6$) or solvents (1 mL 1% bovine serum albumin in solvent; $N = 6$).

Before administration of the next dose of acetylcholine, detaNONOate, papaverine and pinacidil, renal blood flow was allowed to return to baseline values. The doses and timing of administration of the pharmacological agents were determined in pilot experiments. The upper limit of the dose-response curve to acetylcholine, detaNONOate, papaverine and pinacidil was chosen as the highest dose that was devoid of systemic blood pressure effects. The dose of the connexin-mimetic peptides was selected as the lowest dose that achieved the maximal effect. Time-response curves showed that the effect of the peptides was maximal after five minutes and generally disappeared after 30 minutes.

Series 2. The renal blood flow response to intrarenal acetylcholine, detaNONOate, pinacidil and papaverine was examined before and 5 and 30 minutes after infusion of ^{40}Gap 27 peptide ($N = 6$) or control peptide ($N = 6$). The experiments were performed in the absence of systemic NO synthase and cyclooxygenase blockade.

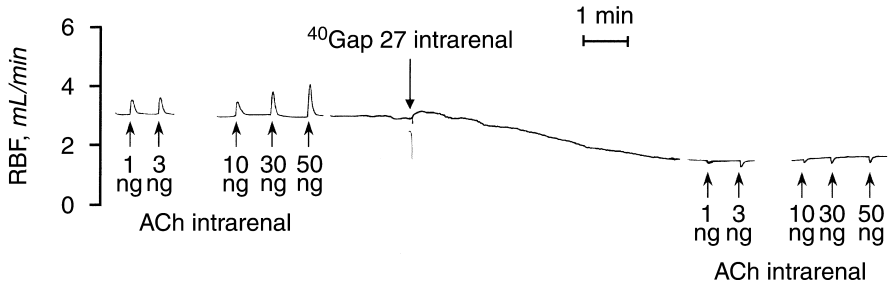

Fig. 1. Representative trace of a dose-response curve to acetylcholine (ACh) before and after administration of ^{40}Gap 27 peptide. The experiment was performed in the continuous and combined presence of systemic nitric oxide (NO) synthase and cyclooxygenase blockade.

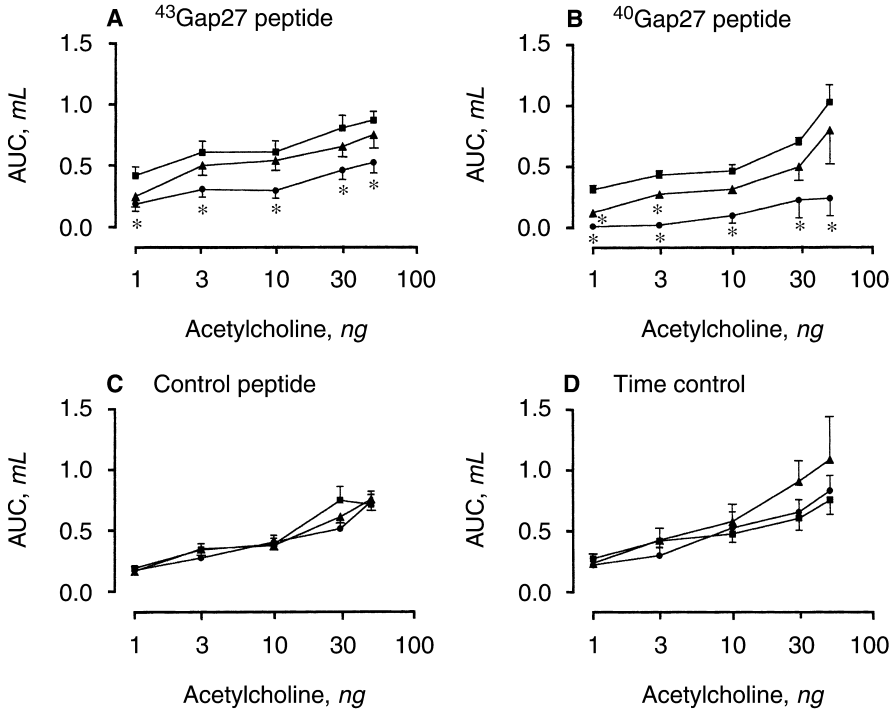

Fig. 2. The renal blood flow increase in response to intrarenal acetylcholine after intravenous L-NAME and indomethacin before (■), 5 min after (●), and 30 min after (▲) intrarenal infusion of a connexin-mimetic peptide. (*A*) ^{43}Gap 27 peptide ($N = 8$), $^*P < 0.05$ vs. baseline (*B*) ^{40}Gap 27 peptide ($N = 6$), $^*P < 0.01$ vs. baseline; (*C*) control peptide ($N = 6$); (*D*) solvent ($N = 6$). The area under the curve (AUC) of the change from baseline values was calculated for each bolus acetylcholine and the data are expressed as mean ± SEM.

Statistical analysis

The data are presented as mean ± SEM. The renal blood flow response to the different agonists is expressed as the area under the curve of the change in renal blood flow (mL/min × min), as detailed previously [18]. Analysis of variance, paired and unpaired *t* tests were used as appropriate. The significance level was set at $P < 0.05$.

RESULTS

Effects on the NO synthase- and cyclooxygenase-independent renal vasodilation to acetylcholine

Intrarenal infusion of ^{43}Gap27, a short peptide possessing conserved sequence homology to part of the second extracellular loop of connexin 43, partially inhibited the L-NAME- and indomethacin-resistant renal blood flow response to acetylcholine, when it was evaluated five minutes after administration of the peptide (Figs. 1 and 2). The renal blood flow response recovered to baseline values, when the dose-response curve to acetylcholine was repeated 30 minutes after infusion of the peptide.

Infusion of ^{40}Gap27, which is homologous to the second extracellular loop of connexin 40, abolished the L-NAME- and indomethacin-resistant renal vasodilatation to acetylcholine five minutes after infusion (Fig. 1). The response almost completely recovered 30 minutes after infusion of the peptide. A control peptide, with a replacement of two animo acids in the motif SRPTEK present in the second extracellular loop of connexins 40 and 43, was without effect on the vasodilation to acetylcholine (Fig. 2). The L-NAME- and indomethacin-resistant vasodilatation to acetylcholine remained stable over time after infusion of solvent without connexin-mimetic peptide (Fig. 2).

Effects on the renal vasodilatation to acetylcholine in the absence of systemic NO synthase- and cyclooxygenase-blockade

In the absence of L-NAME and indomethacin, intrarenal infusion of ^{40}Gap27 decreased the renal vasodilation to acetylcholine five minutes after infusion (Fig. 3). The response to acetylcholine recovered to baseline val-

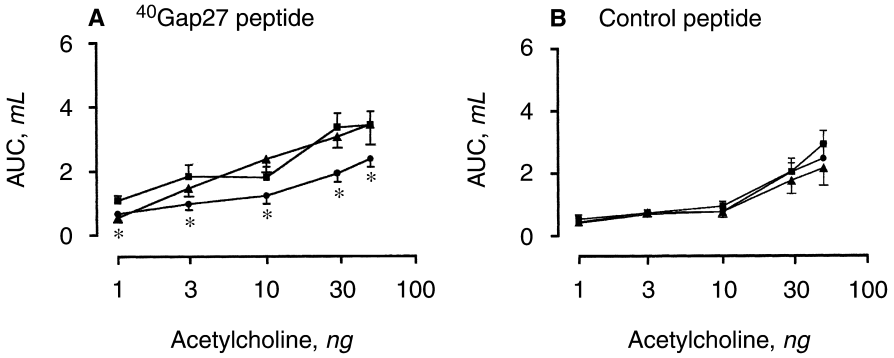

Fig. 3. The renal blood flow increase in response to intrarenal acetylcholine in the absence of L-NAME and indomethacin before (■), 5 min after (●) and 30 min after (▲) intrarenal infusion of a connexin-mimetic peptide. (*A*) ^{40}Gap 27 peptide ($N = 6$), $^{*}P < 0.05$ vs. baseline; (*B*) control peptide ($N = 6$). The area under the curve (AUC) of the change from baseline values was calculated for each bolus of acetylcholine and the data are expressed as mean ± SEM.

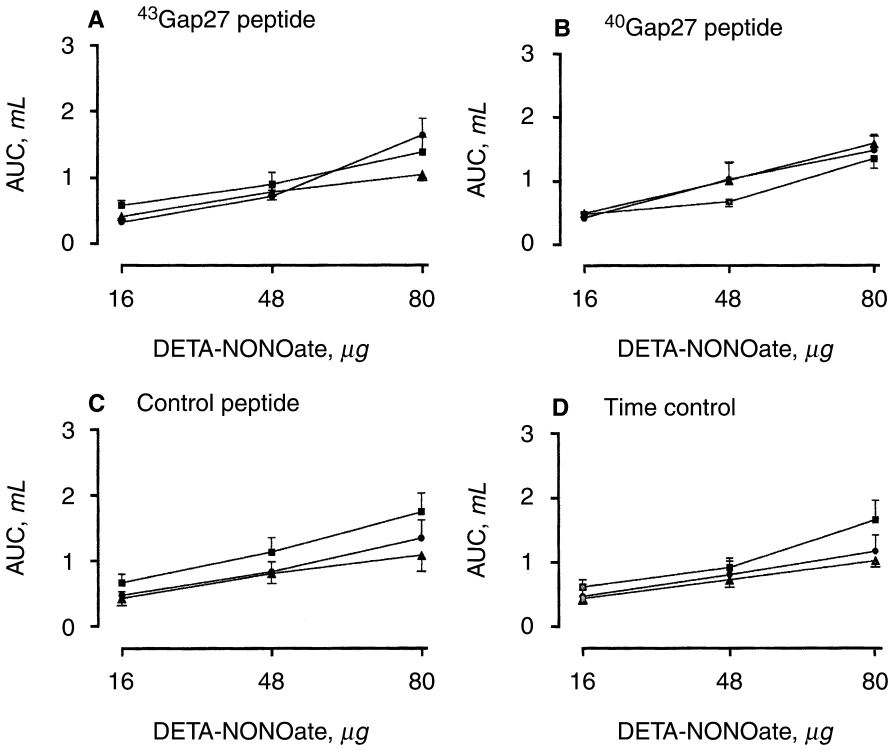

Fig. 4. The renal blood flow increase in response to intrarenal DETA-NONOate after intravenous L-NAME and indomethacin before (■), 5 min after (●) and 30 min after (▲) intrarenal infusion of a connexin-mimetic peptide. (*A*) ^{43}Gap 27 peptide ($N = 8$); (*B*) ^{40}Gap 27 peptide ($N = 6$); (*C*) control peptide ($N = 6$); (*D*) solvent ($N = 6$). The area under the curve (AUC) of the change from baseline values was calculated for each bolus DETA-NONOate and the data are expressed as mean ± SEM.

ues 30 minutes after infusion of the peptide. The control peptide did not affect the renal blood flow response to acetylcholine (Fig. 3).

Effects on the renal vasodilation to DETA-NONOate, pinacidil and papaverine

^{43}Gap27, ^{40}Gap27 and the control peptide did not affect the renal vasodilation to DETA-NONOate, pinacidil and papaverine when tested under conditions of systemic NO synthase and cyclooxygenase blockade (Figs. 4, 5 and 6). The vasodilation to DETA-NONOate, pinacidil and papaverine remained stable over time after infusion of solvent without connexin-mimetic peptide (Figs. 4–6). ^{40}Gap27 and the control peptide did not alter the renal vasodilation to DETA-NONOate, pinacidil and papaverine, measured in the absence of L-NAME and indomethacin (data not shown).

Effects on basal renal blood flow, mean arterial blood pressure and heart rate

Basal renal blood flow was not different between the treatment groups: 4.73 ± 0.21 mL/min (5.31 ± 0.17 mL/min/g kidney weight) in the ^{43}Gap27 group, 4.47 ± 0.25 mL/min (5.30 ± 0.28 mL/min/g kidney weight) in the ^{40}Gap27 group, 4.05 ± 0.19 mL/min (5.04 ± 0.35 mL/min/g kidney weight) in the control peptide group and 4.28 ± 0.33 mL/min (5.14 ± 0.47 mL/min/g kidney weight) in the solvents group ($P > 0.05$ between groups). Renal blood flow decreased significantly after systemic L-NAME and indomethacin infusion and to a similar extent in all treatment groups: 29.5 ± 2.2% in the ^{43}Gap27 group, 30.4 ± 5.0% in the ^{40}Gap27 group, 34.3 ± 3.0% in the control peptide group and 26.7 ± 4.9% in the solvents group ($P > 0.05$ between groups; Fig. 7). Infusion of ^{43}Gap27 caused a mild but significant decrease

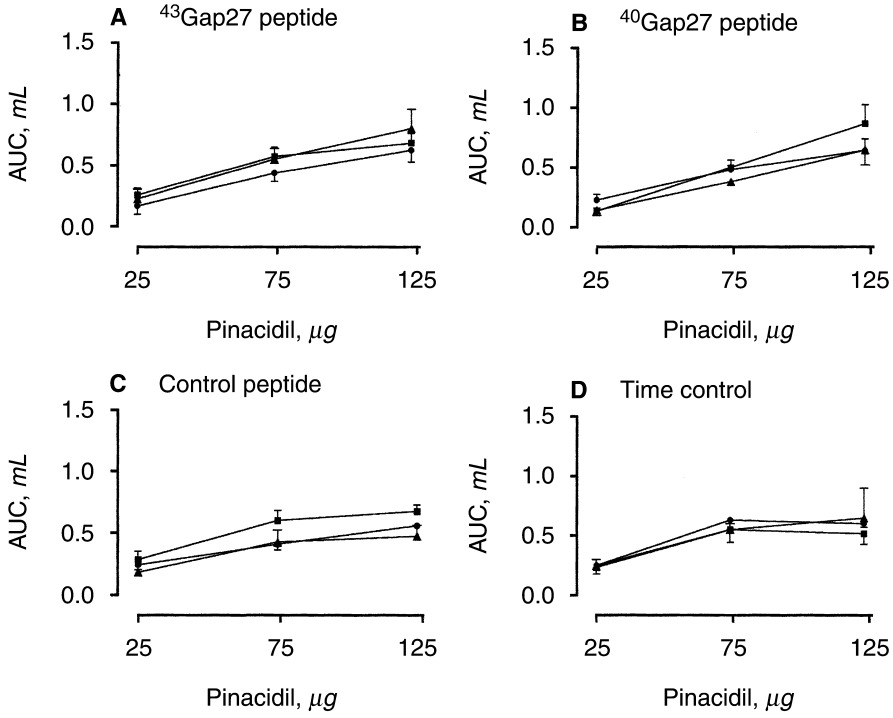

Fig. 5. The renal blood flow increase in response to intrarenal pinacidil after intravenous L-NAME and indomethacin before (■), 5 min after (●) and 30 min after (▲) intrarenal infusion of a connexin-mimetic peptide. (*A*) ^{43}Gap 27 peptide ($N = 8$); (*B*) ^{40}Gap 27 peptide ($N = 6$); (*C*) control peptide ($N = 6$); (*D*) solvent ($N = 6$). The area under the curve (AUC) of the change from baseline values was calculated for each bolus pinacidil and the data are expressed as mean ± SEM.

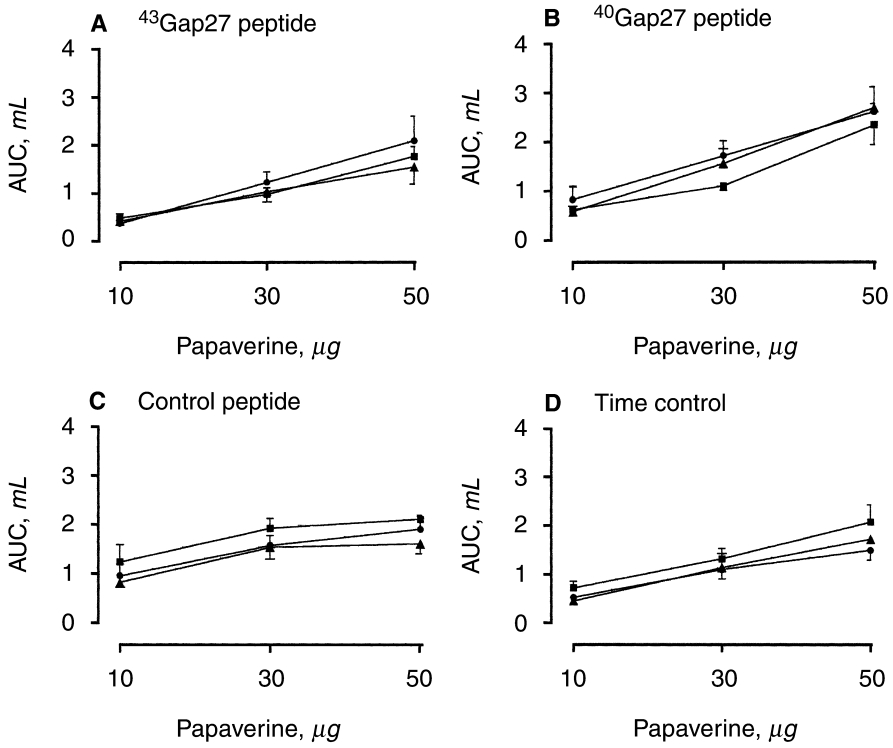

Fig. 6. The renal blood flow increase in response to intrarenal papaverine after intravenous L-NAME and indomethacin before (■), 5 min after (●) and 30 min after (▲) intrarenal infusion of a connexin-mimetic peptide. (*A*) ^{43}Gap 27 peptide ($N = 8$); (*B*) ^{40}Gap 27 peptide ($N = 6$); (*C*) control peptide ($N = 6$); (*D*) solvent ($N = 6$). The area under the curve (AUC) of the change from baseline values was calculated for each bolus papaverine and the data are expressed as mean ± SEM.

of basal renal blood flow, in addition to the fall caused by combined NO synthase- and cyclooxygenase-blockade. After intrarenal administration of ^{40}Gap27, a more pronounced decrease of basal renal blood flow was observed. Thirty minutes after infusion of the peptides, renal blood flow recovered to pre-treatment values. In contrast, infusion of the control peptide or of the solvent did not affect renal blood flow (Fig. 7).

Baseline mean blood pressure was not different between the treatment groups: 123.4 ± 3.7 mm Hg in the

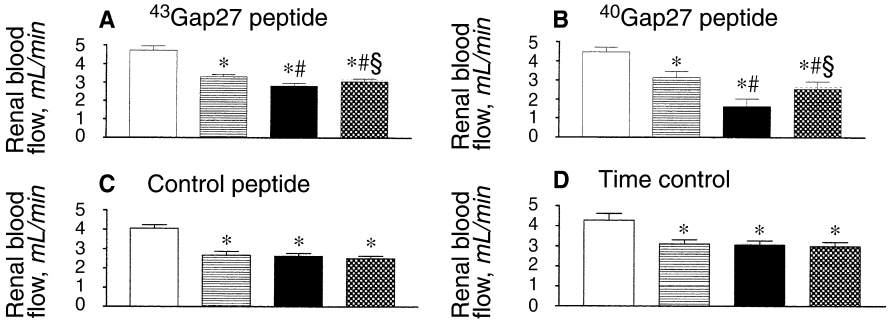

Fig. 7. Renal blood flow (RBF) in baseline conditions (□), after intravenous administration of L-NAME with indomethacin (▤), 5 min after intrarenal infusion of a connexin-mimetic peptide (■) and 30 min after the infusion of the peptide (▩). (*A*) ^{43}Gap 27 peptide ($N = 8$); (*B*) ^{40}Gap 27 peptide ($N = 6$); (*C*) control peptide ($N = 6$); (*D*) solvent ($N = 6$). $*P < 0.001$ vs. baseline values, $\#P < 0.001$ vs. after L-NAME with indomethacin, $\S P < 0.02$ vs. 5 min after the peptide.

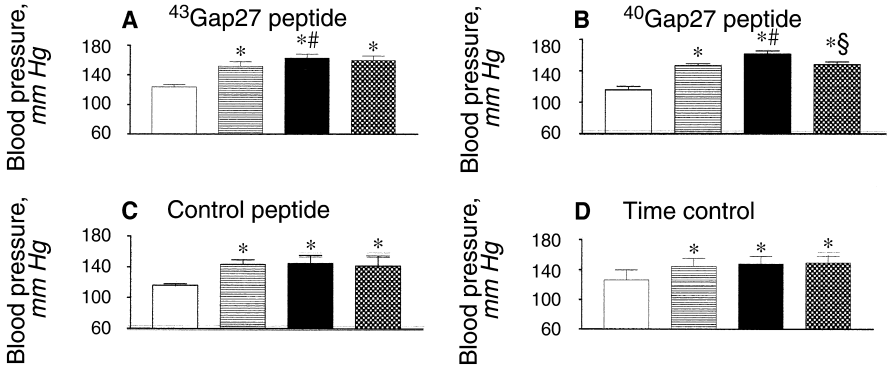

Fig. 8. Mean arterial blood pressure (BP) in baseline conditions (□), after intravenous administration of L-NAME with indomethacin (▤), 5 min after intrarenal infusion of a connexin-mimetic peptide (■), and 30 min after the infusion of the peptide (▩). (*A*) ^{43}Gap 27 peptide ($N = 8$); (*B*) ^{40}Gap 27 peptide ($N = 6$); (*C*) control peptide ($N = 6$); (*D*) solvent ($N = 6$). $*P < 0.01$ vs. baseline values, $\#P < 0.01$ vs. after L-NAME with indomethacin, $\S P < 0.01$ vs. 5 min after the peptide.

^{43}Gap27 group, 115.8 ± 4.3 mm Hg in the ^{40}Gap27 group, 116.0 ± 2.2 mm Hg in the control peptide group, and 125.8 ± 13.6 mm Hg in the solvents group ($P > 0.05$ between groups). Concomitant systemic infusion of L-NAME and indomethacin increased mean arterial blood pressure significantly and to a similar extent in the different treatment groups: 23.4 ± 4.6% in the ^{43}Gap27 group, 26.8 ± 3.5% in the ^{40}Gap27 group, 23.9 ± 6.0% in the control peptide group and 16.5 ± 4.6% in the solvents group ($P > 0.05$ between groups; Fig. 8). Infusion of ^{43}Gap27 caused a mild but significant increase of systemic blood pressure, in addition to the rise caused by combined NO synthase- and cyclooxygenase-blockade. After intrarenal administration of ^{40}Gap27, a significant rise of systemic blood pressure was observed. Thirty minutes after infusion of the peptides, blood pressure fell to values similar to those before administration of the peptides. Infusion of the control peptide or solvents did not affect blood pressure (Fig. 8).

When the experiments were conducted in the absence of L-NAME and indomethacin, intrarenal infusion of ^{40}Gap27 significantly decreased basal renal blood flow after five minutes, with a recovery to baseline values after 30 minutes. Administration of the control peptide did not alter basal renal blood flow (Fig. 9). After infusion of ^{40}Gap27 without L-NAME and indomethacin, a significant increase of mean arterial blood pressure was observed, whereas blood pressure remained stable after infusion of the control peptide (Fig. 10).

The administration of connexin-mimetic peptides did not induce alterations in heart rate: the heart rate was 318 ± 4.9/min and 328 ± 3.7/min before and after ^{40}Gap27, respectively, and 300 ± 13.7/min and 293 ± 11.2/min before and after the control peptide, respectively.

DISCUSSION

In the renal microcirculation of the rat, a large residual response to acetylcholine is present after combined high-dose NO synthase- and cyclooxygenase inhibition, as described previously [18]. A short peptide homologous to a sequence in the second extracellular loop of connexin 40 is a potent inhibitor of the NO synthase- and cyclooxygenase-independent acetylcholine-induced vasodilation in the kidney. A peptide homologous to the extracellular loop of connexin 43 also inhibits the NO synthase- and cyclooxygenase-independent acetylcholine-induced vasodilation, but to a lesser extent. The salient observation made in the present study is that the administration of these connexin-mimetic peptides in vivo substantially decreases basal renal blood flow and increases systemic blood pressure. The results thus suggest that gap junctional communication is essential for EDHF-mediated signal transmission in the kidney in vivo, and provide compelling evidence to support a role for EDHF in the control of blood pressure and tissue perfusion.

Previous studies have shown that connexin-mimetic peptides are capable of interfering with EDHF-mediated signal transmission in rabbit thoracic aorta [20], rabbit

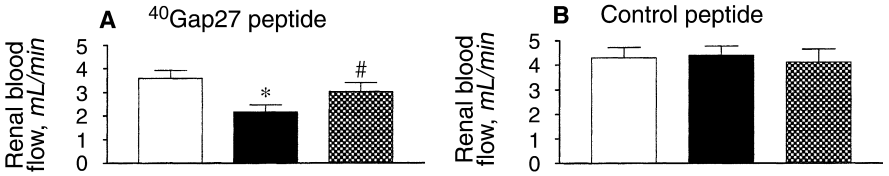

Fig. 9. Renal blood flow (RBF) in baseline conditions (□), 5 min after intrarenal infusion of a connexin-mimetic peptide (■) and 30 min after the infusion of the peptide (▨). (*A*) ^{40}Gap 27 peptide ($N = 6$); (*B*) control peptide ($N = 6$). $*P < 0.01$ vs. baseline values, $\#P < 0.05$ vs. 5 min after the peptide.

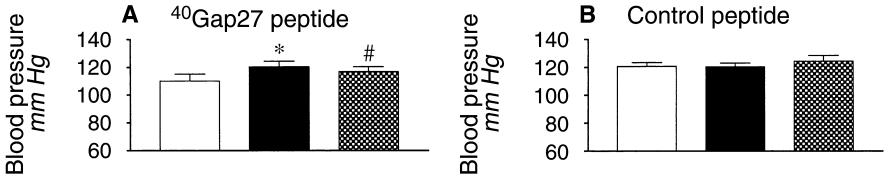

Fig. 10. Mean arterial blood pressure (BP) in baseline conditions (□), 5 min after intrarenal infusion of a connexin-mimetic peptide (■), and 30 min after the infusion of the peptide (▨). (*A*) ^{40}Gap 27 peptide ($N = 6$); (*B*) control peptide ($N = 6$). $*P < 0.01$ vs. baseline values, $\#P < 0.05$ vs. 5 min after the peptide.

superior mesenteric artery [20, 22, 23], rabbit jugular vein [21], guinea pig carotid artery [24], rat hepatic and mesenteric artery [24] and pig coronary artery [25]. In all these studies ^{43}Gap 27 peptide was used, thus suggesting that connexin 43 is the most important connexin for EDHF-mediated responses in these blood vessels. In the present study of the rat renal microcirculation, ^{43}Gap 27 peptide only partially inhibited the L-NAME- and indomethacin-resistant renal vasodilation in response to acetylcholine. However, ^{40}Gap 27 peptide abolished the response, supporting a more important role for connexin 40 in this vascular bed. Both ^{40}Gap 27 and ^{43}Gap 27 contain the motif SRPTEK present in the second extracellular loop of connexins 40 and 43. A control peptide, with a replacement of two amino acids in this motif, was inactive, underlining the specificity of the results. The acetylcholine response recovered to almost normal values 30 minutes after infusion of the ^{40}Gap 27 and ^{43}Gap 27 peptides, suggesting that binding is reversible and that the peptides are removed from the circulation, possibly by glomerular filtration or degradation by proteases. None of the peptides interfered with the responses to DETA-NONOate, pinacidil and papaverine, indicating specificity for endothelium-dependent responses.

Although the present results and those of several in vitro studies [19–25] support the involvement of gap junctions in EDHF-mediated relaxations, the type of information that passes through gap junctions remains enigmatic. In view of the evidence that in some vascular beds EDHF is a humoral substance, myoendothelial gap junctions may represent its site of transfer from the endothelium to the smooth muscle cells. Recently, strong evidence implicated 11,12-EET as an EDHF in porcine coronary arteries [6] and in hamster muscle resistance arteries [7]. Also in renal arteries, it has been suggested that EETs may represent an EDHF [30]. EETs, however, are hydrophobic and therefore in principle are not capable of passing through the aqueous pore of gap junctions. Alternatively, myoendothelial gap junctions may be the site of electrotonic propagation of hyperpolarization from the endothelial cells to the smooth muscle cells [4], obviating the need for a chemical "EDHF."

It should be noted that none of the gap junctional blocking agents studied to date can discriminate between inhibition of myoendothelial gap junctions and inhibition of gap junctional coupling within the endothelial or smooth muscle layers. It is usually assumed that gap junction blockers affect transfer of an "EDHF" from the endothelium to smooth muscle cells via myoendothelial gap junctions. An equally tenable assumption is that they inhibit the propagation of hyperpolarization within the endothelial or the smooth muscle cell layer.

The exploration of the physiological role of EDHF has been hampered by the absence of selective inhibitors of EDHF that are suitable for in vivo use. Several groups have expounded the hypothesis that EDHF may be a back-up vasodilator mechanism in conditions with compromised bioavailability of NO [22, 31, 32]. It can be questioned, however, whether such a complex and powerful mechanism would have developed, only to serve as a reserve vasodilator system. Pulsatile changes in intraluminal pressure induce the synthesis of EDHF, the inhibition of which leads to a marked reduction in vascular compliance [33]. EDHF was found to play a role in the maintenance of basal perfusion pressure in the perfused rat mesenteric arterial bed [34]. In other studies, however, the removal of endothelium did not affect the resting membrane potentials, suggesting that there is no important tonic release of EDHF under basal conditions, at least not in vitro [16]. Charybdotoxin and apamin induced only a minor increase in resistance in the isolated perfused hindlimb of eNOS knockout mice, arguing against a significant basal release of EDHF in that model [31]. The results of the present study strongly support a tonic release of EDHF in vivo, with a modulation of basal vascular tone. Administration of ^{40}Gap 27

peptide, which abolished the L-NAME- and indomethacin-resistant vasodilation induced by acetylcholine, also substantially decreased basal renal blood flow and increased systemic blood pressure. The rise in blood pressure induced by the peptide may result from the increased renal vascular resistance, although spillover of the peptide in the systemic circulation cannot be excluded. As suggested by the absence of changes in heart rate, no major interference with cardiac connexins occurred that could explain the blood pressure effects. A similar decrease in renal blood flow and increase in blood pressure was observed in the absence of L-NAME and indomethacin, underlining that the effects are not merely uncovered by NO synthase- and cyclooxygenase-blockade, and arguing against a back-up role for EDHF. The findings are fully in line with the observation that connexin 40-deficient mice are hypertensive [28].

If EDHF has a role in the control of vascular resistance and tissue perfusion, the findings of impaired EDHF-mediated relaxation in hypertension [35, 36], hypercholesterolemia [37], diabetes mellitus [18] and aging [38] have important implications for the development of vascular complications associated with these risk factors. Conversely, strategies that improve EDHF-release, as demonstrated for folate [18] or nifedipine [39], or mimic it, including the administration of K^+-channel openers, may have potential beneficial therapeutic impact.

In conclusion, inhibition of gap junctional communication with connexin-mimetic peptides blocks EDHF-mediated signal transmission in vivo, as suggested by the abolishment of L-NAME- and indomethacin-resistant renal vasodilation. The peptides also decreased basal renal blood flow and increased blood pressure, supporting a role for tonic EDHF release in the control of tissue perfusion and vascular resistance.

ACKNOWLEDGMENTS

ADV is supported by a grant from the Fund for Scientific Research-Flanders (N20/0). The authors thank Tommy Dheuvaert and Julien Dupont for their expert technical assistance.

Reprint requests to An De Vriese, M.D., Ph.D., Renal Unit, University Hospital, OK12, De Pintelaan 185, B-9000 Gent, Belgium
E-mail: an.devriese@rug.ac.be

REFERENCES

1. Cohen RA, Vanhoutte PM: Endothelium-dependent hyperpolarization. Beyond nitric oxide and cyclic GMP. *Circulation* 92: 3337–3349, 1995
2. Feletou M, Vanhoutte PM: The third pathway: Endothelium-dependent hyperpolarization. *J Physiol Pharmacol* 50:525–534, 1999
3. Feletou M, Vanhoutte PM: Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br J Pharmacol* 93:515–524, 1988
4. Mombouli JV, Vanhoutte PM: Endothelium-derived hyperpolarizing factor(s): Updating the unknown. *Trends Pharmacol Sci* 18: 252–256, 1997
5. Quilley J, McGiff JC: Is EDHF an epoxyeicosatrienoic acid? *Trends Pharmacol Sci* 21:121–124, 2000
6. Fisslthaler B, Popp R, Kiss L, *et al*: Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature* 401:493–496, 1999
7. 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
8. Randall MD, Kendall DA: Endocannabinoids: A new class of vasoactive substances. *Trends Pharmacol Sci* 19:55–58, 1998
9. Edwards G, Dora KA, Gardener MJ, *et al*: K^+ is an endothelium-derived hyperpolarizing factor in rat arteries. *Nature* 396:269–272, 1998
10. Popp R, Bauersachs J, Hecker M, *et al*: A transferable, beta-naphthoflavone-inducible, hyperpolarizing factor is synthesized by native and cultured porcine coronary endothelial cells. *J Physiol* 497:699–709, 1996
11. Chen G, Yamamoto Y, Miwa K, Suzuki H: Hyperpolarization of arterial smooth muscle induced by endothelial humoral substances. *Am J Physiol* 260:H1888–H1892, 1991
12. Little TL, Xia J, Duling BR: Dye tracers define differential endothelial and smooth muscle coupling patterns within the arteriolar wall. *Circ Res* 76:498–504, 1995
13. Sandow SL, Hill CE: Incidence of myoendothelial gap junctions in the proximal and distal mesenteric arteries of the rat is suggestive of a role in endothelium-derived hyperpolarizing factor-mediated responses. *Circ Res* 86:341–346, 2000
14. Nagao T, Illiano S, Vanhoutte PM: Heterogeneous distribution of endothelium-dependent relaxations resistant to N^G-nitro-L-arginine in rats. *Am J Physiol* 263:H1090–H1094, 1992
15. Hwa JJ, Ghibaudi L, Williams P, Chatterjee M: Comparison of acetylcholine-dependent relaxation in large and small arteries of rat mesenteric vascular bed. *Am J Physiol* 266:H952–H958, 1994
16. Shimokawa H, Yasutake 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
17. 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
18. De Vriese AS, Van de Voorde J, Blom HJ, *et al*: The impaired renal vasodilator response attributed to endothelium-derived hyperpolarizing factor in streptozotocin-induced diabetic rats is restored by 5-methyltetrahydrofolate. *Diabetologia* 43:1116–1125, 2000
19. Taylor HJ, Chaytor AT, Evans WH, Griffith TM: Inhibition of the gap junctional component of endothelium-dependent relaxations in rabbit iliac artery by 18-alpha glycyrrhetinic acid. *Br J Pharmacol* 125:1–3, 1998
20. Chaytor AT, Evans WH, Griffith TM: Central role of heterocellular gap junctional communication in endothelium-dependent relaxations of rabbit arteries. *J Physiol* 508:561–573, 1998
21. Griffith TM, Taylor HJ: Cyclic AMP mediates EDHF-type relaxations of rabbit jugular vein. *Biochem Biophys Res Commun* 263: 52–57, 1999
22. Hutcheson IR, Chaytor AT, Evans WH, Griffith TM: Nitric oxide-independent relaxations to acetylcholine and A23187 involve different routes of heterocellular communication. Role of gap junctions and phospholipase A2. *Circ Res* 84:53–63, 1999
23. Dora KA, Martin PEM, 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
24. Edwards G, Feletou M, Gardener MJ, *et al*: Role of gap junctions in the responses to EDHF in rat and guinea-pig small arteries. *Br J Pharmacol* 128:1788–1794, 1999
25. Edwards G, Thollon C, Gardener MJ, *et al*: Role of gap junctions and EETs in endothelium-dependent hyperpolarization of porcine coronary artery. *Br J Pharmacol* 129:1145–1154, 2000
26. Kumar NM, Gilula NB: The gap junction communication channel. *Cell* 84:381–388, 1996
27. Christ GJ, Spray DC, el-Sabban M, *et al*: Gap junctions in vascular tissues. Evaluating the role of intercellular communication in the modulation of vasomotor tone. *Circ Res* 79:631–646, 1996
28. de Wit C, Roos F, Bolz SS, *et al*: Impaired conduction of vasodila-

tion along arterioles in connexin 40-deficient mice. *Circ Res* 86: 649–655, 2000
29. Campbell WB, Harder DR: Endothelium-derived hyperpolarizing factors and vascular cytochrome P450 metabolites of arachidonic acid in the regulation of tone. *Circ Res* 84:484–488, 1999
30. Fleming I, Busse R: Vascular cytochrome P450 in the regulation of renal function and vascular tone: EDHF, superoxide anions and blood pressure. *Nephrol Dial Transplant* 16:1309–1311, 2001
31. Brandes RP, Schmitz-Winnenthal FH, Feletou M, *et al*: An endothelium-derived hyperpolarizing factor distinct from NO and prostacyclin is a major endothelium-dependent vasodilator in resistance vessels of wild-type and endothelial NO synthase knockout mice. *Proc Natl Acad Sci USA* 97:9747–9752, 2000
32. Kilpatrick EV, Cocks TM: Evidence for differential roles of nitric oxide (NO) and hyperpolarization in endothelium-dependent relaxation of pig isolated coronary artery. *Br J Pharmacol* 112:557–565, 1994
33. Popp R, Fleming I, Busse R: Pulsatile stretch in coronary arteries elicits release of endothelium-derived hyperpolarizing factor: A modulator of arterial compliance. *Circ Res* 82:696–703, 1998
34. Adeagbo AS, Triggle CR: Varying extracellular [K^+]: A functional approach to separating EDHF- and EDNO-related mechanisms in perfused rat mesenteric arterial bed. *J Cardiovasc Pharmacol* 21:423–429, 1993
35. Van de Voorde J, Vanheel B, Leusen I: Endothelium-dependent relaxation and hyperpolarization in aorta from control and renal hypertensive rats. *Circ Res* 70:1–8, 1992
36. Fujii K, Tominaga M, Ohmori S, *et al*: Decreased endothelium-dependent hyperpolarization to acetylcholine in smooth muscle of the mesenteric artery of spontaneously hypertensive rats. *Circ Res* 70:660–669, 1992
37. Eizawa H, Yui Y, Inoue R, *et al*: Lysophosphatidylcholine inhibits endothelium-dependent hyperpolarization and N omega-nitro-L-arginine/indomethacin-resistant endothelium-dependent relaxation in the porcine coronary artery. *Circulation* 92:3520–3526, 1995
38. Fujii K, Ohmori S, Tominaga M, *et al*: Age-related changes in endothelium-dependent hyperpolarization in the rat mesenteric artery. *Am J Physiol* 265:H509–H516, 1993
39. Fisslthaler B, Hinsch N, Chataigneau T, *et al*: Nifedipine increases cytochrome P4502C expression and endothelium-derived hyperpolarizing factor-mediated responses in coronary arteries. *Hypertension* 36:270–275, 2000