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Loss of endothelial K_{ATP} channel-dependent, NO-mediated dilation of endocardial resistance coronary arteries in pigs with left ventricular hypertrophy

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1 The influence of left ventricular hypertrophy (LVH) on the endothelial function of resistance endocardial arteries is not well established. The aim of this study was to characterise the mechanisms responsible for UK-14,304 (α_2-adrenoreceptor agonist)-induced endothelium-dependent dilation in pig endocardial arteries isolated from hearts with or without LVH.

2 LVH was induced by aortic banding 2 months before determining endothelial function. Following euthanasia, hearts were harvested and endocardial resistance arteries were isolated and pressurised to 100 mmHg in no-flow conditions. Vessels were preconstricted with acetylcholine (ACh) or high external K^+ (40 mmol l^{-1} KCl). Results are expressed as mean ± s.e.m.

3 UK-14,304 induced a maximal dilation representing 79 ± 6% ($n = 8$) of the maximal diameter. NO synthase (L-NNA, 10 μmol l^{-1}, $n = 7$) or guanylate cyclase (ODQ, 10 μmol l^{-1}, $n = 4$) inhibition reduced ($P < 0.05$) UK-14,304-dependent dilation to 35 ± 6 and 18 ± 7%, respectively. Apamin and charybdotoxin reduced ($P < 0.05$) to 39 ± 8% ($n = 4$) the dilation induced by UK-14,304. In depolarised conditions, however, this dilation was prevented ($P < 0.05$).

4 UK-14,304-induced dilation was reduced ($P < 0.05$) by glibenclamide (Glib, 1 μmol l^{-1}), a K_{ATP} channel blocker, either alone (35 ± 10%, $n = 5$) or in combination with L-NNA (34 ± 9%, $n = 4$).

5 In LVH, UK-14,304-induced maximal dilation was markedly reduced (25 ± 4%, $P < 0.05$) compared to control; it was insensitive to L-NNA (21 ± 5%) but prevented either by the combination of L-NNA, apamin and charybdotoxin, or by 40 mmol l^{-1} KCl.

6 Activation of endothelial α_2-adrenoreceptor induces an endothelium-dependent dilation of pig endocardial resistance arteries. This dilation is in part dependent on NO, the release of which appears to be dependent on the activation of endothelial K_{ATP} channels. This mechanism is blunted in LVH, leading to a profound reduction in UK-14,304-dependent dilation.
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Abbreviations: ACh, acetylcholine; Apa, apamin; BK, bradykinin; Chtx, charybdotoxin; E_{max}, maximal dilation; EDHF, endothelium-derived hyperpolarizing factor; Glib, glibenclamide; indo, indomethacin; K_{ATP}, ATP-sensitive K^+ channels; K_{Ca}, Ca^{2+}-sensitive K^+ channel; L-NNA, *N*ω-nitro-L-arginine; LVH, left ventricular hypertrophy; MT, myogenic tone; NO, nitric oxide; ODQ, 1H-[1,2,4]Oxadiazolo[4,3a]quinoxaline-1-one; PGI_2, prostacyclin; PSS, physiological salt solution; SNP, sodium nitroprusside

Introduction

Left ventricular hypertrophy (LVH) is the main morphological adaptive change of the heart in response to a chronic increase in pressure. LVH is a common clinical finding affecting 23% of men and 33% of women over the age of 59 (Savage *et al*., 1987). LVH is characterised by an increased myocardial mass, which requires an increase in coronary blood flow to maintain function. Compression of the microvasculature and lack of blood supply, however, may be associated with myocardial ischemia (Pichard *et al*., 1981; Marcus *et al*., 1982; Opherk *et al*., 1984). In patients with LVH, arteriolar density was found to be identical to that of healthy controls, whereas arteriolar medial area and peri-arteriolar collagen area were increased (Mundhenke *et al*., 1997). Although such structural changes may partly account for the impaired coronary reserve in LVH (Kozakova *et al*., 2000; Rajappan *et al*., 2002; 2003), additional vascular changes must be involved.

Endothelial dysfunction, a common feature of vascular diseases, is characterised by a reduced dilatory capacity (Taniyama & Griendling, 2003). Endothelium-dependent dilation is reduced in patients with LVH, in addition to coronary flow reserve, whereas the minimum coronary resistance is increased (Treasure *et al*., 1992; 1993; Egashira *et al*., 1995; Houghton *et al*., 1998; 2003; Kozakova *et al*., 2000; Di Bello *et al*., 2002; Rajappan *et al*., 2002; 2003). As previously shown in human (Houghton *et al*., 1998), we reported in pigs that the endothelium-dependent relaxation of large epicardial coronary arteries was impaired in LVH

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following 2 months of aortic banding (Malo *et al*., 2003). Although free radicals are implicated in all cardiovascular diseases (for review, Taniyama & Griendling, 2003), the cellular mechanisms leading to this dysfunction are unclear. In addition, it is unclear whether endothelial microvascular function is similarly altered. Our previous data (Thorin, 2001) and those of others (Thollon *et al*., 1999) demonstrate that cardiovascular diseases do not alter endothelial function in general, but affect particular receptor systems. To address this issue, we have investigated the mechanisms of action of α_2-adrenergic receptor (α_2-AR)-mediated NO-dependent dilation of pressurised endocardial resistance arteries isolated from healthy pigs and pigs with LVH. The results demonstrate that NO production is not impaired in LVH; however, α_2-AR coupling leading to NO release is blunted.

Methods

Animals

In total, 70 2-month-old male Landrace swine (25 ± 3 kg) were maintained and tested in accordance with the recommendations of the guidelines on the care and use of laboratory animals issued by the Canadian Council on Animal Research and the guidelines of the Animal Care, and were approved by the local committee. Two experimental groups were studied: a control group, which consisted of swine killed by exsanguination without aortic banding and a LVH group, with aortic banding for a period of 60 days before killing.

Surgical procedure

Induction of LVH was performed by aortic banding as previously described (Malo *et al*., 2003). Briefly, anaesthesia was induced by intramuscular injection of ketamine ($20\,mg\,kg^{-1}$) and xylazine ($2\,mg\,kg^{-1}$). It was supported by halothane 1% $v\,v^{-1}$ during artificial ventilation with an O_2/air mixture. Arterial blood gases were monitored. Acidosis was balanced with 8.4% sodium. A catheter was placed in an auricular vein for intravenous infusion during operation. A femoral catheter was placed to monitor arterial pressure and for arterial blood sampling. A left thoracotomy was performed in the third intercostal space. The ascending aorta and the pulmonary vein were carefully separated. An umbilical tape was placed 3 cm above the coronary ostia of the aorta, gently constricted to obtain a negative systolic gradient of 15 mmHg (measured by monitoring the systemic arterial pressure distally to the banding site) and then tied. The pericardium was loosely closed and the chest was closed in multiple layers.

Sacrifice and coronary microvessels harvesting

In total, 4-month-old sham swine (Control, 40 ± 2 kg) and LVH (42 ± 2 kg) swine with 60 days of aortic banding were anesthetised using the same approach as for the surgical procedure. After exsanguination, the thorax was reopened through a median sternotomy to excise the heart, which was immediately placed in cold (4°C) physiological salt solution (PSS) of the following composition ($mmol\,l^{-1}$): NaCl 130; KCl 4.7; $CaCl_2$ 1.6; $MgSO_4$ 1.17; $NaHCO_3$ 14.9; KH_2PO_4 1.18; EDTA 0.026; glucose 10; and oxygenated with 12% O_2/5% CO_2/83% N_2 (pH 7.4). Hearts were weighed and a section of the left ventricle was isolated and placed in a Petri dish containing cold PSS supplemented with indomethacin ($10\,\mu mol\,l^{-1}$, cyclooxygenase inhibitor). Subendocardial coronary microvessels (100–250 μm in external diameter) were dissected out from the anterior-free wall of the left ventricle.

Morphometric analysis

Subendocardial arterioles (100–250 μm external diameter) were dissected with a myocardial block, fixed in formalin 10% and cut transversely. Slices were stained with Verhoeff. Total vessel area and lumen area were determined by digital planimetry using a video microscope linked to a computer and a customised software (Scion image 1.6, Frederick, MD, U.S.A.). Wall area was determined by subtraction of the lumen area from the total area. In order to obtain comparable data at variable diameters, the wall to lumen ratio was calculated.

Coronary microvessel studies

Vessels were placed in a 2 ml plexiglass organ chamber containing oxygenated PSS warmed at 37°C, cannulated with dual glass micropipettes, and secured with 10–0 nylon monofilaments sutures as previously described (Véquaud & Thorin, 2001). Vessels were pressurised to 100 mmHg in a no-flow state. This internal pressure was selected based on preliminary experiments demonstrating that within a range of 80–120 mmHg, myogenic responses were optimal in the two groups of vessels. Myogenic tone ($n = 7$) represented 18 ± 3, 20 ± 4 and 19 ± 4% at 80, 100 and 120 mmHg in the control group, and 11 ± 4, 14 ± 4 and 17 ± 4% at 80, 100 and 120 mmHg in the LVH group. With an inverted microscope connected to a video camera, the vessel image was projected onto a black and white monitor. A video dimension analyzer (Living Systems Intrumentation, Burlington, VT, U.S.A.) was used to acquire online internal lumen diameter. Vessels were allowed to equilibrate in the organ chamber for 50–60 min before initiation of the experimental protocols.

Study protocols

After equilibration, the arterial segments were constricted by extraluminal addition of acetylcholine (ACh; $10\,\mu mol\,l^{-1}$), which induced a reduction in diameter representing 75% of the maximal diameter, that is, $44 \pm 5\,\mu m$ from $188 \pm 10\,\mu m$ in control and $50 \pm 5\,\mu m$ from $205 \pm 5\,\mu m$ in LVH vessels. Single cumulative concentration–response curve was obtained, either to UK-14,304 ($1\,pmol\,l^{-1}$ to $0.3\,\mu mol\,l^{-1}$), to bradykinin (BK; $1\,fmol\,l^{-1}$ to $0.3\,\mu mol\,l^{-1}$), or to sodium nitroprusside (SNP; $1\,nmol\,l^{-1}$ to $0.3\,mmol\,l^{-1}$). All drugs were applied extraluminally. At the end of the protocol, the maximal diameter (D_{max}) was determined by changing the PSS to a Ca^{2+}-free PSS containing SNP ($10\,\mu mol\,l^{-1}$) and EGTA (ethylene glycol-bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, $1\,mmol\,l^{-1}$).

In another series of experiments, vessels were constricted by a $40\,mmol\,l^{-1}$ KCl-PSS depolarising solution added to the organ chamber. Depolarisation induced a reduction in diameter representing 75% of the maximal diameter, that is, $49 \pm 7\,\mu m$ from $199 \pm 14\,\mu m$ in control and $60 \pm 10\,\mu m$ from $222 \pm 8\,\mu m$ in LVH vessels. Single cumulative concentration–

response curve to the agonist was obtained, followed by the determination of the D_{max}, as described above.

All experiments were performed in the presence of indomethacin ($10\,\mu mol\,l^{-1}$). Inhibitors were added in the bath (extraluminally) after the equilibration period and 20 min before initiation of the experiment (dilation to one agonist on ACh-induced constriction). NO formation was inhibited by *N*ω-nitro-L-arginine (L-NNA; $10\,\mu mol\,l^{-1}$), an inhibitor of NO synthase. Cyclic GMP formation was inhibited by 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxaline-1-one (ODQ, $10\,\mu mol\,l^{-1}$), an inhibitor of the soluble guanylate cyclase. Glibenclamide ($1\,\mu mol\,l^{-1}$) was used to block ATP-dependent potassium channels (K_{ATP}). Finally, to further confirm the involvement of a putative endothelium-derived hyperpolarizing factor (EDHF), apamin ($1\,\mu mol\,l^{-1}$) and charybdotoxin ($0.1\,\mu mol\,l^{-1}$) were used to block calcium-activated potassium channels (K_{Ca}), the small conductance K_{Ca} (SK_{Ca}) and the intermediate conductance K_{Ca} (IK_{Ca})/big conductance K_{Ca} (BK_{Ca}), respectively.

Drugs

Acetylcholine, bradykinin, UK-14,304, sodium nitroprusside, indomethacin, *N*ω-nitro-L-arginine, 1H-[1,2,4]Oxadiazolo[4,3a]quinoxaline-1-one (ODQ), glibenclamide, apamin and charybdotoxin were obtained from Sigma Chemical Co., St Louis, MO, U.S.A. All drugs were dissolved in ultrapure distilled water except for glibenclamide, which was dissolved in DMSO as a stock solution ($10\,mmol\,l^{-1}$) and dissolved in PSS to obtain the final dilution ($1\,\mu mol\,l^{-1}$), and indomethacin, which was dissolved in ethanol as a stock solution ($10\,mmol\,l^{-1}$). Stock solutions were stored at $-20^{\circ}C$. Dilutions were prepared daily.

Data analysis

In every case, *n* refers to the number of animals used in each protocol. Half-maximum effective concentration (EC_{50}) of each drug was measured from each individual concentration–response curve using a logistic curve-fitting program (Allfit, Dr DeLéan, Université de Montréal, Montréal, QC, Canada). The pD_2 value, the negative log of the EC_{50}, was obtained. Continuous variables are expressed as mean ± standard error of the mean (s.e.m.). For each protocol, basal diameter in no-flow condition was determined at the end of the 50-min equilibration period. Myogenic tone, which is a reduction in diameter induced by an increase in luminal pressure, was measured and expressed as percentage of the D_{max}. Agonist-induced dilation is expressed as a percentage of the D_{max}. ANOVA were performed to compare concentration–response curves. Differences were considered to be statistically significant when the *P*-value was <0.05 (Scheffe's F-test).

Results

Baseline parameters

In total, 60 days of aortic banding led to an increase ($P<0.05$) in heart to body weight ratio ($6.8\pm0.9\,g\,kg^{-1}$) compared to the control group ($5.7\pm1.3\,g\,kg^{-1}$). The haemodynamic characteristics of LVH in this model have been published (Malo *et al.*, 2003). Briefly, left diastolic and end-diastolic intraventricular pressures were increased and relaxation of the left ventricle was impaired. In addition, morphometric and haemodynamic parameters did not differ between control and sham-operated animals (Malo *et al.*, 2003).

The wall to lumen ratio of resistance arteries (100–250 μm external diameter) increased from 1.5 ± 0.1 ($n=6$) in control vessels to 2.3 ± 0.3 ($n=8$) in LVH.

Isolated and pressurised endocardial arteries developed myogenic tone (Table 1). This reduction in diameter induced by the intraluminal pressure was not different between the two groups. Whereas pharmacological inhibition of the NO synthase had no influence on the myogenic tone of arteries isolated from control hearts, it increased myogenic tone ($P<0.05$) in vessels isolated from LVH hearts. In contrast, blockade of K_{ATP} channel with glibenclamide increased ($P<0.05$) myogenic tone in control but not LVH vessels. Guanylate cyclase inhibition with ODQ augmented myogenic tone more ($P<0.05$) in arteries from control than LVH hearts, whereas dual inhibition of the NO pathway and the K_{Ca} channels increased myogenic tone similarly in both groups (Table 1). In vessels isolated from control pigs, apamin and charybdotoxin alone increased myogenic tone to $57\pm8\%$ of D_{max} ($n=4$).

UK-14,304 induced dilation

The α_2-AR agonist UK-14,304 induced an endothelium-dependent dilation of pressurised coronary microvessels. This dilation (Table 2) was reduced ($P<0.001$) in the LVH group when compared to controls (Figure 1). The dilatation was insensitive to L-NNA in the LVH group, whereas addition of L-NNA impaired ($P<0.001$) the maximal dilation of control arteries. The guanylate cyclase inhibitor ODQ reduced ($P<0.05$) the dilation of control vessels to a level comparable to that induced by L-NNA (Table 2).

UK-14,304-induced dilation was abolished in both controls and LVH groups under depolarised conditions (high external K^+). When present, L-NNA only partially prevented UK-14,304-induced responses (Figure 1), suggesting an additional mechanism of action by the latter that may be independent of NO synthesis. To test whether the actions of UK-14,304 may be due to depolarisation, dilations induced by this compound were examined in the presence of glibenclamide ($1\,\mu mol\,l^{-1}$).

Table 1 Myogenic tone of pressurised coronary endocardial arteries

Experimental conditions	*Control*	n	*LVH*	n
PSS	13 ± 3	23	8 ± 2	17
+ L-NNA	14 ± 6	7	33 ± 11*	5
+ Glib	36 ± 7*	9	9 ± 5†	5
+ ODQ	75 ± 5*	10	46 ± 13*,†	6
+ L-NNA + Apa + Chtx	63 ± 25*	3	51 ± 14*	3

Myogenic tone (% D_{max}) was measured in the absence (PSS) or 20 min after preincubation of the tissues with *N*ω-nitro-L-arginine (+ L-NNA, $10\,\mu mol\,l^{-1}$), glibenclamide (Glib, $1\,\mu mol\,l^{-1}$), 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxaline-1-one (ODQ, $10\,\mu mol\,l^{-1}$) or a combination of L-NNA, apamin (Apa, $1\,\mu mol\,l^{-1}$) and charybdotoxin (Chtx, $0.1\,\mu mol\,l^{-1}$). Data are mean ± s.e.m. All experiments were performed in the presence of indomethacin ($10\,\mu mol\,l^{-1}$). *$P<0.05$ compared to PSS; $^{\dagger}P<0.05$ compared to Control.

Table 2 Pharmacological parameters for UK-14,304-induced dilation of pressurised coronary endocardial arteries

UK, 14 304	*Control*			*LVH*		
	pD_2	E_{max} (%)	n	pD_2	E_{max} (%)	n
PSS	10.1±0.3	79±6	8	10.0±0.1	25±4†	5
+L-NNA	10.2±0.3	35±6*	7	9.8±0.4	21±5	5
+40 mmol l^{-1} KCl	ND	0±0*	5	ND	3±1*	5
+Glib	10.9±0.7	35±10*	5	ND	ND	
+ODQ	9.9±0.7	18±7*	4	ND	ND	
+Apa+Chtx	7.9±0.2*	39±8*	4	ND	ND	
+L-NNA+Glib	9.6±0.7	34±9*	4	ND	ND	
+L-NNA+Apa+Chtx	ND	3±1*	3	ND	2±1*	3

Vascular sensitivity (pD_2) and maximal dilation (E_{max}) to the α_2-AR agonist were measured in arteries isolated from control pigs and pigs with left ventricular hypertrophy (LVH) following 2 months of aortic banding. Data are mean±s.e.m. All experiments were performed in the presence of indomethacin (10 μmol l^{-1}). In the absence of KCl-PSS, arterioles were preconstricted with acetylcholine (10 μmol l^{-1}). For drug concentrations, please refer to the section '*Study protocols*'. *$P<0.001$ compared to PSS; †$P<0.001$ compared to Control. ND: Not determined.

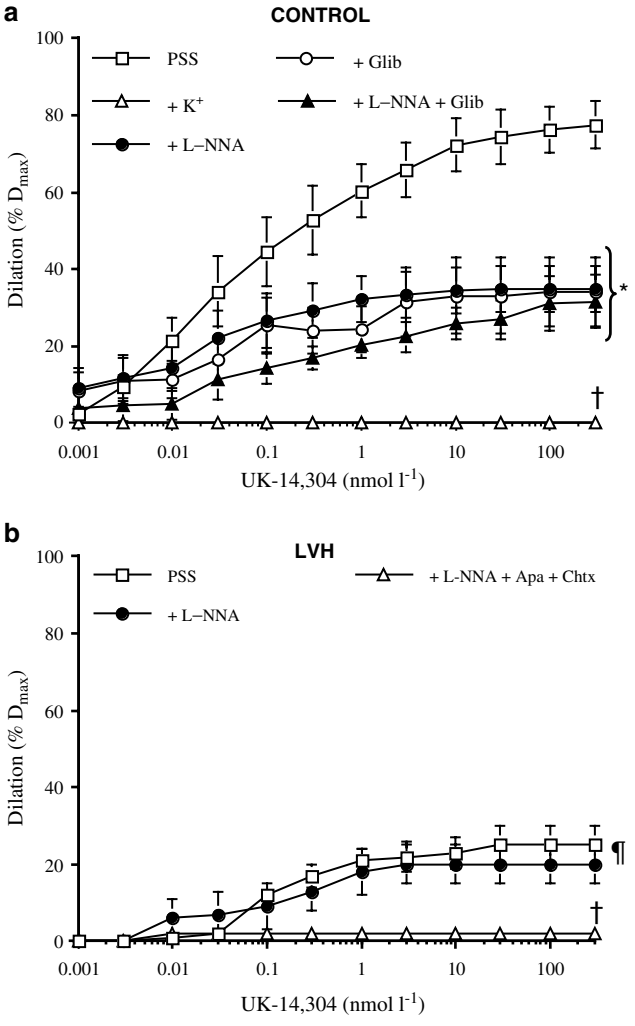

Figure 1 UK-14,304-induced dilation of isolated and pressurised endocardial coronary arteries isolated from (a) control and (b) LVH pigs. Experiments were performed in the absence (PSS) or presence of a depolarising solution (+K^+, 40 mmol l^{-1} KCl PSS), *N*ω-nitro-L-arginine (+L-NNA, 10 μmol l^{-1}), glibenclamide (Glib, 1 μmol l^{-1}) or a combination of L-NNA and glibenclamide or L-NNA and apamin (1 μmol l^{-1})+charybdotoxin (0.1 μmol l^{-1}). Data are mean±s.e.m. of six to seven independent experiments. All solutions contained indomethacin *$P<0.05$ compared to PSS and with K^+; †$P<0.05$ compared to all groups; ¶$P<0.05$ compared to PSS Control (a).

As shown in Table 2, glibenclamide produced similar inhibitions to L-NNA. The actions of these two inhibitors were not additive (Figure 1a).

To confirm the involvement of a putative EDHF in the L-NNA- and indomethacin-resistant dilation induced by UK-14,304, apamin (1 μmol l^{-1}) and charybdotoxin (0.1 μmol l^{-1}) were added to the organ bath. In the presence of the two K_{Ca} channel inhibitors, UK-14,304-induced dilation was reduced by half in arteries isolated from control hearts (Table 2). In combination with L-NNA, UK-14,304-induced dilation was prevented in both groups (Table 2).

Bradykinin-induced dilation

BK-induced dilation was similar in the control and LVH group (Table 3). In the presence of high external K^+, the concentration–response curve to BK was shifted to the right, whereas the maximal dilation induced by BK was slightly reduced in the control ($P<0.05$) but not LVH group. In the presence of L-NNA, BK-induced dilation was unaltered and similar in both groups. Addition of ODQ in the presence of both indomethacin and 40 mmol l^{-1} K^+-PSS abolished the dilation to BK in both groups (Table 3). Glibenclamide did not affect BK-induced dilation (Table 3).

Endothelium-independent dilation

SNP-induced endothelium-independent dilation was similar in control and LVH coronary microvessels (Table 3). In depolarised conditions, vascular sensitivity and potency of SNP were not altered. ODQ, however, abolished ($P<0.001$) the dilatory response to SNP in arteries isolated from both groups (Table 3). Glibenclamide had no influence on the vascular potency and efficacy of SNP (Table 3).

Discussion

The objectives of this study were to investigate the mechanisms of action of NO-induced dilation of pressurised coronary endocardial resistance arteries of healthy pigs and pigs with LVH induced by 2 months of aortic banding. Our data suggest that NO-induced dilation depends on endothelial K_{ATP}-channels opening when stimulated by an α_2-AR agonist. In

Table 3 Pharmacological parameters for bradykinin (BK)- and sodium nitroprusside (SNP)-induced dilation of pressurised coronary endocardial arteries.

BK	*Control*			*LVH*		
	pD_2	E_{max} (%)	n	pD_2	E_{max} (%)	n
PSS	11.0±0.3	97±2	8	10.7±0.7	101±4	5
+Glibenclamide	10.6±0.4	96±3	3	9.2±0.1	96±2	3
+L-NNA	9.6±0.4*	90±4	5	9.5±0.2	95±4	5
+40 mmol l^{-1} KCl	9.7±0.2*	83±4*	7	10.0±0.3	93±1	8
+KCl+ODQ	ND	3±2*	3	ND	2±2*	3
SNP	*Control*			*LVH*		
	pD_2	E_{max} (%)	n	pD_2	E_{max} (%)	n
PSS	5.7±0.3	83±8	7	4.8±0.3	66±9	7
+40 mmol l^{-1} KCl	5.1±0.2	72±4	8	4.6±0.1	83±10	5
+Glib	5.5±0.3	76±11	4	4.5±0.4	50±3	5
+ODQ	ND	4±3*	3	ND	2±2*	3

Vascular sensitivity (pD_2) and maximal dilation (E_{max}) were measured in arteries isolated from control pigs and pigs with left ventricular hypertrophy (LVH) following 2 months of aortic banding. Data are mean±s.e.m. All experiments were performed in the presence of indomethacin (10 μmol l^{-1}). In control conditions, arterioles were preconstricted with acetylcholine (10 μmol l^{-1}). For drug concentrations, please refer to the section '*Study protocols*'. *$P<0.001$ compared to PSS. ND: Not determined.

LVH, this mechanism is blunted and not compensated by a secondary endothelium-dependent pathway. We also found that endothelium-dependent dilation to BK and endothelium-independent dilation to SNP do not depend on K_{ATP}-channels opening, and are not affected by LVH.

Addition of L-NNA, in the presence of indomethacin, reduced by ≈50% the maximal dilation to UK-14,304 in arteries isolated from control pigs. On the other hand, combined inhibition of IK_{Ca} and BK_{Ca} with apamin and charybdotoxin reduced by ≈50% the maximal dilation to UK-14,304. Hence, both NO and an apamin- and charybdotoxin-sensitive factor, presumably EDHF (Edwards *et al*., 1998; Thollon *et al*., 2002), contribute to UK-14,304-induced dilation in arterioles isolated from control animals. Interestingly, however, 40 mmol l^{-1} KCl-PSS alone abolished the UK-14,304-induced dilation. This full block obtained in depolarising conditions suggested to us that a K^+-dependent mechanism contributed to UK-14304-mediated, NO-dependent dilation.

It has been demonstrated that NO can interact directly with different K^+ channels such as ATP-sensitive K^+ channels (K_{ATP}), BK_{Ca} and the voltage-gated K^+ channel (K_v) (Bolotina *et al*., 1994; Miyoshi *et al*., 1994; Peng *et al*., 1996; Yuan *et al*., 1996; Li *et al*., 1997; Bychkov *et al*., 1998). These effects depend upon both the type of blood vessel and the species studied. For instance, NO can directly activate K_{Ca} in smooth muscle cell membrane patches (inside-out configuration) isolated from the rabbit aorta in the absence of cGMP (Bolotina *et al*., 1994). Others have reported that adenosine can open endothelial K_{ATP} channels through activation of a *pertussis* toxin-sensitive G_i protein. The endothelial hyperpolarisation increases the inward driving force for Ca^{2+}, which leads to the production and release of NO (Hein & Kuo, 1999). Similar to adenosine, endothelial α_2-AR is coupled to G_i proteins and leads to vascular dilation (Véquaud & Thorin, 2001).

In order to verify that K_{ATP} channels are involved in α_2-AR-dependent NO-induced dilation, we used glibenclamide. This reduced the maximal dilation as efficiently as L-NNA. Furthermore, combination of both L-NNA and glibenclamide did not further reduce the maximal dilation. This strongly supports the concept that L-NNA and glibenclamide blocked the same pathway. Hence, K_{ATP} channels are implicated in NO induced dilation. There are two alternative pathways: first, UK-14,304-dependent K_{ATP} channel activation leads to a rise in intracellular Ca^{2+} responsible for NOS activation (Hein & Kuo, 1999). Second, UK-14,304-dependent phospholipase C activation leads to the release of NO, which activates smooth muscle K_{ATP} channels through a cGMP-dependent mechanism (Murphy & Brayden, 1995). Our protocols did not allow for discrimination between the two pathways. However, the following results are suggestive of a direct α_2-AR-dependent activation of endothelial K_{ATP} channels:

- High external K^+ did not prevent BK-induced dilation of arterioles isolated from control pigs. This demonstrates that, in contrast to UK-14,304-induced dilation, (1) cGMP-dependent NO dilation (sensitive to ODQ) is able to fully compensate EDHF inhibition and (2) NO does not activate smooth muscle K_{ATP} channels to induce dilation as revealed by the lack of effect of glibenclamide on BK-induced dilation;
- As for BK, SNP-induced dilation was not affected under depolarising conditions with or without K_{ATP} channel inhibition. As expected, the dilation was prevented by ODQ, demonstrating that SNP-induced dilation is NO-mediated and cGMP-dependent.

Based on these findings, it is likely that α_2-AR-dependent activation of endothelial K_{ATP} channels accounts for NO production and release. Upon reaching the smooth muscle, NO stimulates the soluble guanylate cyclase, which triggers dilation. It is unlikely indeed that NO targets smooth muscle K_{ATP} channels only when endothelial α_2-AR are activated. Furthermore, and as discussed above, apamin and charybdotoxin reduced UK-14,304-induced dilation but did not abolish it. This is in agreement with the concept that endothelial K_{Ca} channels accounts for EDHF-mediated dilation (Edwards *et al*., 1998; Thollon *et al*., 2002), a mechanism distinct from K_{ATP} channel-dependent NO production (Hein & Kuo, 1999).

Effect of LVH on UK-14,304 induced dilation

In arterioles isolated from pigs with LVH, the maximal dilation induced by UK-14,304 was severely impaired when compared to control. This endothelial dysfunction became apparent during α_2-AR stimulation. No significant reduction in BK-induced dilation was observed. The lack of impairment of BK-mediated relaxation has been previously reported in pig coronary arteries 4 weeks postangioplasty (Thollon *et al*., 1999). Similarly, we previously reported that substance P-induced relaxation of coronary arteries from atherosclerotic patients was not reduced whereas ACh induced a contraction (Thorin, 2001). Hence, there is no global endothelial dysfunction in LVH, but rather an impairment of selected pathways.

Inhibition of NO synthase did not further reduce the dilation induced by UK-14,304, demonstrating that the α_2-AR-dependent pathway leading to NO production is altered, probably sparing the EDHF pathway (L-NNA- and indomethacin-resistant component). We confirmed that the remaining dilation was only attributable to a putative EDHF since the dilation was sensitive to a depolarising solution or to the combination of apamin and charybdotoxin, two K_{Ca} channel blockers known to block this pathway (Adeagbo & Triggle, 1993; Edwards *et al*., 1998; Beny & Schaad, 2000; Brandes *et al*., 2000; Véquaud & Thorin, 2001; Thollon *et al*., 2002). The limitation of our approach is the absence of membrane potential determination to confirm that repolarisation of the smooth muscle is responsible for the apamin- and charybdotoxin-sensitive dilation.

The origin of this selective inhibition of the endothelial α_2-AR pathway is unknown. In a previous study, we reported that α_2-AR activation led to the release of NO through the activation of the PLC by the $\beta\gamma$-subunits of the G_i protein, whereas activation of a putative K^+ current was linked to the α_i-subunit (Véquaud & Thorin, 2001). It is known that in pathological conditions leading to endothelial dysfunction, G_i but not G_q proteins are dysfunctional (Perrault *et al*., 1999). It is possible that in LVH, similar selective alterations occur.

Myogenic tone is a reduction in diameter induced by an increase in intraluminal pressure. Although myogenic in nature, this contraction is largely influenced by the endothelium (Nguyen *et al*., 1999), as revealed by the rise in myogenic tone after inhibition of both NO and EDHF production by L-NNA combined with apamin and charybdotoxin (Table 1). LVH, however, influenced myogenic tone regulation by the endothelium. In the presence of L-NNA, myogenic tone augmented, a result not observed in control vessels. This remains an observation for which more studies are required. Either the importance of constitutively released NO is augmented in LVH, or the rise in the production of an endothelium-derived constricting factor, such as endothelin-1, is unmasked by NO withdrawal (Nguyen *et al*., 1999; Thorin *et al*., 1999; Kinlay *et al*., 2001). On the other hand, glibenclamide had no effect in vessels isolated from hearts with LVH, which supports the concept that these channels are malfunctioning in this pathological setting.

Clinical significance

Previous work by Bache's group demonstrated that, in comparison with normal hearts, hypertrophied hearts have increased reliance on opening of K_{ATP} channels to augment coronary blood flow during exercise (Melchert *et al*., 1999). This study demonstrated that, contrary to the normal heart, adenosine was not mandatory for exercise-induced coronary dilation. In addition, Lavallée's group reported that the stimulation of endothelial β_2-AR involved the opening of K_{ATP} channels (Ming *et al*., 1997). Our data suggest therefore that these mechanisms would be impaired in LVH.

In conclusion, our data suggest that endothelium-derived NO induces a dilation that primarily depends on the opening of endothelial K_{ATP}-channels when stimulated by UK-14,304, an α_2-AR agonist. In LVH, this mechanism of action is blunted and not compensated by a secondary endothelium-dependent pathway. The impact of this pathway on the regulation of the perfusion of the endocardium still remains to be demonstrated.

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