Changes in EDHF in hypertension and ageing: response to chronic treatment with renin-angiotensin system inhibitors

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Summary

1. Endothelial function is impaired in hypertension and ageing and this may be associated with an increase in cardiovascular disease. Several clinical studies have shown that blocking the renin-angiotensin system (RAS) improves endothelial function not only in hypertensive patients but also in normotensive patients with cardiovascular disease. The aim of the present study was to test whether endothelium-derived hyperpolarising factor (EDHF) -mediated smooth muscle hyperpolarisation and relaxation are altered in hypertension and ageing, and if so, whether chronic treatment with RAS inhibitors (the angiotensin-converting enzyme inhibitor enalapril and the angiotensin type 1 receptor antagonist candesartan) would correct such changes.

2. EDHF-mediated responses were examined in mesenteric arteries from 12-month-old spontaneously hypertensive rats (SHR) and 3-, 6-, 12-, and 24-month-old normotensive Wistar-Kyoto rats (WKY). Furthermore, both strains were treated for three months with either RAS blockers or a conventional therapy with hydralazine and hydrochlorothiazide from 9- to 12-month-old. In arteries of 12-month-old SHR, EDHF-mediated responses were impaired compared with age-matched WKY. In SHR, all the antihypertensive treatments improved the impairment of EDHF-mediated responses; however, RAS inhibitors tended to improve these responses to a greater extent compared with the conventional therapy with hydralazine and hydrochlorothiazide. In arteries of WKY, EDHF-mediated responses were impaired at the age of 12 and 24 months compared with those of 3- and 6-month-old rats, with the response tending to be impaired to a greater extent in 24-month-old rats. Three months of treatment of WKY until the age of 12 months with RAS inhibitors but not with a conventional therapy with hydralazine and hydrochlorothiazide improved the age-related impairment of EDHF-mediated responses, despite a similar reduction in blood pressure by both treatments.

3. These findings suggest that: (1) EDHF-mediated hyperpolarisation and relaxation decline with hypertension and ageing in rat mesenteric arteries; (2) antihypertensive treatment restores the impaired EDHF-mediated responses in hypertension; (3) RAS inhibitors may be more efficacious in improving endothelial dysfunction associated with hypertension; and (4) chronic treatment with RAS inhibitors improves the age-related impairment of EDHF-mediated responses presumably through the blockade of RAS but not blood pressure lowering alone.

Introduction

Endothelial cells play an important role in the regulation of vascular tone through the release of several factors such as nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarising factor (EDHF).[1,2] Although the nature of EDHF is still controversial, EDHF appears to be a dominant vasodilator in resistance arteries.[3-5]

Endothelial dysfunction is associated with various cardiovascular risk factors, such as hypertension, ageing, diabetes mellitus, and hypercholesterolemia.[6,7] Endothelial dysfunction may facilitate the progress of atherosclerosis[6,7], thereby leading to cardiovascular diseases.[8] It is, therefore, of clinical importance to find out the underlying mechanisms of, and effective treatments for endothelial dysfunction. In the present paper, the role of EDHF in hypertension and ageing and its modulation by drug treatment – especially the effects of renin-angiotensin system (RAS) inhibitors – will be discussed.

EDHF in hypertension

Endothelium-dependent relaxation is impaired both in animal models of experimental hypertension and in patients with hypertension.[9] Several mechanisms have been proposed to explain the endothelial dysfunction in hypertension: reduced NO production, increased production of endothelium-derived contracting factors and increased generation of oxygen-derived free radicals.[9]

Fujii *et al.*[10] have evaluated the relative contribution of EDHF in acetylcholine (ACh) -induced responses in the superior mesenteric arteries of spontaneously hypertensive rats (SHR). In this study, they showed that EDHF-mediated hyperpolarisation and relaxation were decreased in SHR compared with age-matched normotensive Wistar-Kyoto rats (WKY). In contrast, endothelium-dependent relaxation via NO was preserved in SHR.[11] Fujii *et al.* have also showed that neither NO synthase inhibitors nor a cyclooxygenase inhibitor affected ACh-induced hyperpolarisation in the rat superior mesenteric arteries,[10] which suggests that ACh-induced hyperpolarisation is not mediated by endothelium derived NO or prostanoids in this vascular bed. Subsequent studies[11-14] confirmed the impairment of EDHF-mediated responses in mesenteric arteries from genetically hypertensive rats. Similar

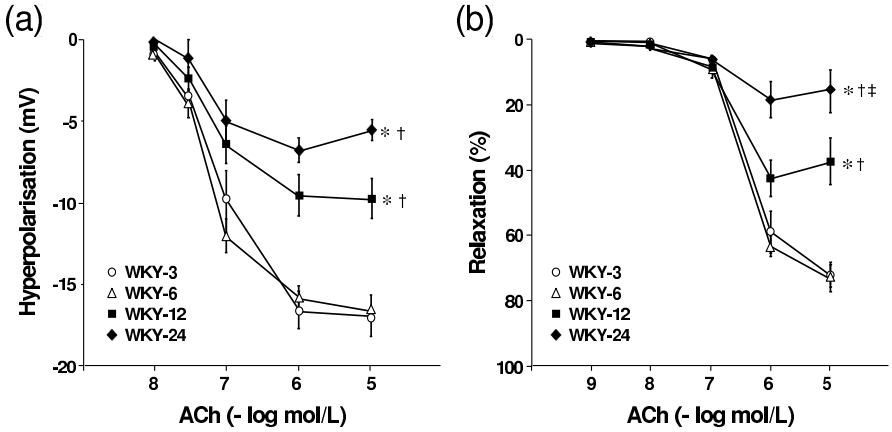

Figure 1. *(a) Acetylcholine (ACh)-induced hyperpolarisation in mesenteric arteries of 3- (WKY-3), 6- (WKY-6), 12- (WKY-12), and 24-month-old Wistar Kyoto rats (WKY-24). ACh was applied under resting conditions without treatment. (b) ACh-induced relaxation in mesenteric arterial rings precontracted with norepinephrine (10^{-5} mol/L) in the presence of indomethacin (10^{-5} mol/L) and N^G-nitro-L-arginine (10^{-4} mol/L) of WKY-3, WKY-6, WKY-12, and WKY-24. Values are mean±SEM (n=6-10). *P<0.05 vs. WKY-3; †P<0.05 vs. WKY-6; ‡P<0.05 vs. WKY-12. (Reproduced from Fujii* et al.,[21] *with permission).*

observations were also reported in the aorta of two-kidney, one clip renal hypertensive rats[15] and in the renal artery of aged SHR.[16] These findings indicate that EDHF-mediated responses are impaired in hypertension, and the impairment of EDHF pathway may account, at least in part, for the endothelial dysfunction associated with hypertension. On the other hand, it has been recently reported that enhanced EDHF effect may compensate for the loss of NO and maintain the vasodilatory response to ACh in mesenteric arteries of Sprague-Dawley rats fed a high salt diet.[17] Furthermore, Sandow *et al.* have reported that the incidence of myoendothelial gap junctions, which enables electrical and/or chemical coupling between endothelial cell and smooth muscle cell layers, was increased to maintain a functional role for EDHF in caudal artery of SHR.[18]

The reason for the difference in the results of these studies is not known, but may in part arise from differences in the type, severity and/or duration of hypertension.

EDHF in ageing

Ageing is associated with endothelial dysfunction both in humans and animal models.[19] Reduced NO-mediated relaxation and/or increased cyclooxygenase-dependent constriction could partially underpin age-related endothelial dysfunction depending on the species and the vascular bed studied.[19] In the present study, age related changes in EDHF-mediated hyperpolarisation and relaxation to ACh were studied in the superior mesenteric arteries from 3-, 6-, 12-, and 24-month-old WKY.[20,21] EDHF-mediated hyperpolarisation was significantly smaller in arteries from 12- and 24-month-old rats compared with 3- and 6-month-old rats, with the response tending to be smaller in 24-month-old rats than in 12-month-old rats. EDHF-mediated relaxation also decreased with increasing age (Fig. 1). In contrast, there was no difference in NO-mediated relaxation between 3- and 12-month-old rats. The age-related decline in EDHF-mediated responses observed here are consistent with previous studies by others.[12,22] Thus, the impairment of the EDHF pathway may account, at least in part, for the age-related endothelial dysfunction in rat mesenteric arteries.

The EDHF pathway does exist in human arteries.[23,24] Urakami-Harasawa *et al.*[24] have reported that EDHF-mediated relaxation was reduced with ageing in human gastroepiploic arteries. Thus, the reduced EDHF-mediated responses would also contribute to the age-related endothelial dysfunction in humans.

Effect of antihypertensive treatment on EDHF-mediated responses in hypertension

Hypertension is associated with endothelial dysfunction.[9] Endothelial dysfunction may aggravate the progression of atherosclerosis, which could lead to cardiovascular disease.[6-8] Hence, it is plausible to suggest that the improvement of endothelial function will reduce the occurrence of cardiovascular disease. Although several studies found that antihypertensive treatments improve endothelial function both in animal models of experimental hypertension[9] and in patients with hypertension,[25] the effects of chronic antihypertensive treatment on EDHF-mediated hyperpolarisation *per se* are unknown.

The effects of chronic antihypertensive treatments on EDHF-mediated hyperpolarisation and relaxation were tested in the mesenteric arteries of SHR.[11,14] SHR were

Table. *Systolic blood pressure before and after 3 months of treatment. Values are mean ± SEM. There were 7 to 12 rats in each group.*

	Blood pressure (mmHg)				Blood pressure (mmHg)	
	Before	After			Before	After
SHR-12	241±6	253±6		WKY-12	150±4	156±5
SHR-12-H	242±6	163±6 *†		WKY-12-H	158±4	124±4 *§
SHR-12-ENA	245±5	135±6 *†		WKY-12-ENA	157±3	123±6 *§
SHR-12-CAN	239±7	120±6 *†‡		WKY-12-CAN	153±3	125±2 *§
SHR-12-C&E	246±7	111±3 *†‡				
WKY-12	151±5 †	155±4 †				

* $P<0.05$ vs before treatment; † $P<0.05$ vs SHR-12; ‡ $P<0.05$ vs SHR-12-H; § $P<0.05$ vs WKY-12

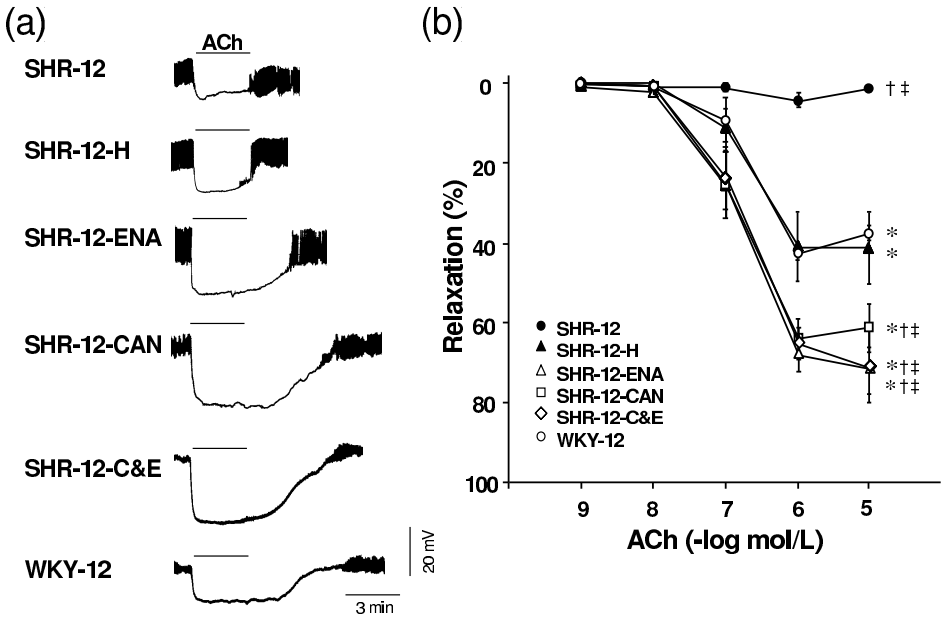

Figure 2. *(a) Representative tracings showing hyperpolarisation to 10^{-5} mol/L acetylcholine (ACh) under conditions of depolarisation with norepinephrine (10^{-5} mol/L) in the presence of indomethacin (10^{-5} mol/L) in mesenteric arteries of untreated 12-month-old spontaneously hypertensive rats (SHR-12), SHR treated with a combination of hydralazine and hydrochlorothiazide (SHR-12-H), enalapril-treated SHR (SHR-12-ENA), candesartan-treated SHR (SHR-12-CAN), SHR treated with a combination of candesartan and enalapril (SHR-12-C&E), and untreated 12-month-old Wistar Kyoto rats (WKY-12). (b) ACh-induced relaxation in mesenteric arterial rings precontracted with norepinephrine (10^{-5} mol/L) in the presence of indomethacin (10^{-5} mol/L) and N^G-nitro-L-arginine (10^{-4} mol/L) of SHR-12, SHR-12-H, SHR-12-ENA, SHR-12-CAN, SHR-12-C&E, and WKY-12. Values are mean±SEM (n=8-12). *P<0.05 vs. SHR-12; †P<0.05 vs. WKY-12; ‡P<0.05 vs. SHR-12-H. (Modified from Goto* et al.,[14] *with permission).*

treated for 3 months with either the combination of hydralazine and hydrochlorothiazide, enalapril, an angiotensin converting enzyme (ACE) inhibitor, candesartan, an angiotensin type 1 (AT1) receptor antagonist, or the combination of enalapril and candesartan from 9- to 12-month-old. The combination of hydralazine and hydrochlorothiazide improved EDHF-mediated hyperpolarisation and relaxation to a similar level to that of WKY. Interestingly, however, the improvement achieved by RAS inhibitors was significantly greater than that with a conventional therapy with hydralazine and hydrochlorothiazide, despite a similar, or only a slightly greater reduction in blood pressure (Table, Fig. 2). These results suggest that in addition to blood pressure lowering, inhibition of the RAS may play an important role in improving endothelial function.[11,14]

Although both ACE inhibitors and AT1 receptor antagonists inhibit the RAS, each drug has its specific

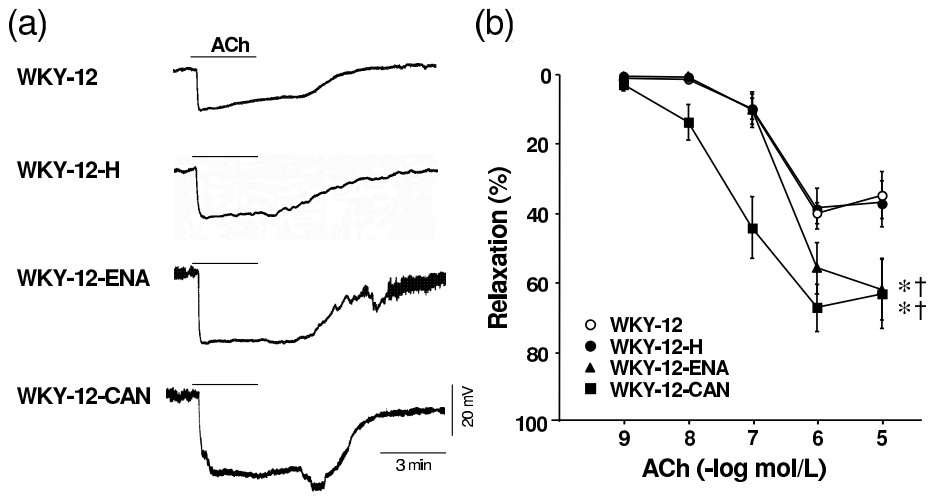

Figure 3. *(a) Representative tracings showing hyperpolarisation to 10^{-5} mol/L acetylcholine (ACh) under conditions of depolarisation with norepinephrine (10^{-5} mol/L) in the presence of indomethacin (10^{-5} mol/L) in mesenteric arteries of untreated 12-month-old Wistar Kyoto rats (WKY-12), WKY treated with a combination of hydralazine and hydrochlorothiazide (WKY-12-H), enalapril-treated WKY (WKY-12-ENA), and candesartan-treated WKY (WKY-12-CAN). (b) ACh-induced relaxation in mesenteric arterial rings precontracted with norepinephrine (10^{-5} mol/L) in the presence of indomethacin (10^{-5} mol/L) and N^G-nitro-L-arginine (10^{-4} mol/L) of WKY-12, WKY-12-H, WKY-12-ENA, and WKY-12-CAN. Values are mean±SEM (n=6-12). * P<0.05 vs. WKY-12; † P<0.05 vs. WKY-12-H. (Modified from Goto* et al.,[31] *with permission).*

pharmacological profiles: ACE inhibitors prevent the degradation of bradykinin, a peptide that induces endothelium-dependent relaxation[23]; AT1 receptor antagonists block the action of angiotensin II regardless of its generation pathway[26]; under blockade of AT1 receptors, angiotensin II may stimulate unopposed angiotensin type 2 receptors.[27] However, in the present study, enalapril and candesartan were equally effective in improving EDHF-mediated responses, which indicates that the specific pharmacological profiles of each drug may not play a major role in improving EDHF-mediated responses in rat mesenteric arteries. Kähönen *et al.*[28] also showed that an ACE inhibitor and an AT1 receptor antagonist improved the EDHF-mediated relaxation to a similar extent in mesenteric arteries of SHR.

Several recent clinical studies[29,30]have reported the beneficial effects of the combination therapy with an ACE inhibitor and an AT1 receptor antagonist. In the present study, however, the combination therapy did not appear to have definitive advantages over each therapy in improving EDHF-mediated responses (Fig. 2).

In summary, the above data indicate that: (1) chronic antihypertensive treatments restore the impaired EDHF-mediated responses in SHR; (2) RAS inhibitors may be more effective in improving endothelial dysfunction; and (3) the combination of an ACE inhibitor and an AT1 receptor antagonist does not seem to be more effective than treatment with either drug alone. The clinical relevance of the present finding remains to be determined.

Effect of renin-angiotensin system inhibitors on EDHF-mediated responses in ageing

Endothelial dysfunction associated with ageing may contribute in part to the frequent occurrence of cardiovascular disease with ageing in humans. Thus, it is clinically relevant to prevent or reverse endothelial dysfunction associated with ageing. In SHR, antihypertensive treatments with RAS inhibitors tended to be more effective in improving endothelial dysfunction compared with conventional antihypertensive drugs.[11,14] These observations led to the hypothesis that RAS inhibitors may have a favourable effect on endothelial function independent of its blood pressure lowering effect.

The effects of RAS inhibitors on age-related impairment of EDHF-mediated responses were studied using mesenteric arteries of WKY.[31,32] WKY were treated for 3 months with either enalapril, candesartan or a combination of hydralazine and hydrochlorothiazide from 9- to 12-month-old. All the treatments lowered blood pressure to a similar extent (Table). EDHF-mediated hyperpolarisation and relaxation were improved in the enalapril and candesartan treated groups. In contrast, a combination of hydralazine and hydrochlorothiazide failed to improve endothelial function, despite a similar reduction in blood pressure (Fig. 3). These findings suggest that RAS inhibitors restore the age-related impairment of EDHF-mediated responses presumably through the blockade of the RAS *per se*, although we cannot totally rule out the possibility that both RAS inhibition and blood pressure

lowering are required for the improvement of endothelial function. Thus, RAS inhibitors may serve as novel tools with which to prevent endothelial dysfunction associated with ageing.

Future directions

Because of the unidentified nature of EDHF,[3-5] the mechanism of the alteration in EDHF associated with hypertension and ageing remains speculative. Likewise, how RAS inhibitors improve impaired EDHF-mediated responses remains an open question. However, considering the critical role of gap junctions in EDHF-mediated responses in rat mesenteric arteries[33,34], impairment of the EDHF pathway and its improvement by RAS inhibitors could be associated with structural and/or biochemical changes in gap junctions. This notion may be supported by the recent report by Rummery *et al.*[35]that showed expression of connexins, which comprise gap junctions, were decreased in the endothelium of the caudal artery in hypertension. Whether impairment of EDHF-mediated responses in disease states is attributable to abnormalities of gap junctions awaits further studies.

Conclusions

EDHF mediated hyperpolarisation and relaxation were impaired in hypertension and ageing. Chronic treatment with RAS inhibitors restored these impairments, and RAS inhibitors appear to have a favourable effect on endothelial function beyond its blood pressure lowering effect. Thus, RAS inhibitors may have a therapeutic potential in the prevention or treatment of cardiovascular diseases.

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