

ORIGINAL ARTICLE

Enhanced vasodilator responses to calcitonin gene-related peptide (CGRP) in subcutaneous arteries in human hypertension

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Isolated segments (1–2 mm) of small subcutaneous arteries (diameter 0.1–0.9 mm) and veins (0.1–1.0 mm) from patients with hypertension (essential $n = 13$, renovascular $n = 6$) and controls ($n = 17$) were examined. The relaxant responses to the sensory transmitters calcitonin gene-related peptide (CGRP) and substance P, and the contractile responses to potassium and noradrenaline were studied. Enhanced dilatory responses (E_{max}) but no change in sensitivity (pEC_{50}) were demonstrated in the arteries but not in the veins to CGRP in hypertensives ($P < 0.01$) as compared with normotensives, and in the hypertensive subgroups (essential hypertension, $P < 0.05$; renovascular hypertension, $P < 0.05$). The relaxant responses to substance P were not

altered either in arteries or in veins of hypertensives. Furthermore, there were no differences in the contractile responses to 60 mM potassium or to 10 μ M noradrenaline between the groups. The results suggest that the enhanced vasodilator response to CGRP in hypertension is an adaptive reaction. The elevated blood pressure may be augmented by vasodilatory activity since different subgroups of hypertensives showed the same results. However, other common characteristics of hypertension (eg, medication, metabolic disturbances) may have also influenced the results.

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Introduction

Calcitonin gene-related peptide (CGRP) is produced by the tissue-specific alternative splicing of the primary transcript of the calcitonin/CGRP gene.¹ This 37 amino-acid peptide is widely distributed in perivascular nerves and is a potent vasodilator.^{2–4} Systemic administration of CGRP decreases blood pressure in a dose-dependent manner in normotensive animals and in humans^{5–7} as well as in spontaneously hypertensive rats (SHR).⁸ The primary mechanism for the reduction in blood pressure is peripheral arterial dilatation.⁷ Therefore, it has been postulated that CGRP plays a role in the modulation of blood pressure under normal conditions and in the pathophysiology of hypertension.

The role of CGRP in hypertension is unclear. Contradictory results have been demonstrated in

human hypertension concerning circulating CGRP. Increased⁹ as well as decreased or unchanged^{10–12} levels of CGRP have been reported in human hypertension. Interestingly, in an animal model of essential hypertension with SHR, the studies have revealed an increase in CGRP-induced vasodilatation as compared with control Wistar-Kyoto (WKY) rats both *in vitro*¹³ and *in vivo*.⁸

The CGRP-induced vasomotor effect is mediated by a complex receptor (for review see Juaneda *et al*¹⁴). A receptor-associated membrane protein (RAMP) interacts with a calcitonin receptor-like receptor (CRLR). The RAMPs facilitate the intracellular translocation of CGRP-maturing protein and its insertion into the plasma membrane. There are at least three different types of RAMPs. RAMP1, upon its co-transfection with CRLR, confers a CGRP₁-receptor profile. Furthermore, an intracellular protein, the receptor component protein (RCP), ensures CGRP receptor signal transduction. Thus, RAMP1, CRLR and RCP association form a high-affinity receptor coupled to G_s to promote intracellular production of cAMP.¹⁴

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Substance P is found in sensory nerves and induces vasodilatation (endothelium-dependent). Intravenous injection elicits a reduction of blood pressure and tachycardia.¹⁵ In human hypertension a blunted vasorelaxant effect to substance P has been shown.^{16–18} However, unaltered endothelium-dependant vasodilatory effect in human hypertension has also been reported.¹⁹ Studies of different classes of SHR seem to indicate that attenuated vasodilatory responses are present^{20,21} and reduced circulating levels of substance-P have been shown.²² In guinea pigs, however, no attenuation of the vasodilatory effect of substance P was demonstrated.²³

Circulating levels of substance P in human hypertension are unaltered,^{10,24} decreased^{25,26} or increased.^{27,28} Furthermore, medication (prazosin, captopril) may increase circulating levels of substance P in human hypertension.²⁶ Enalapril did not increase the circulating levels of substance P.²⁹ Moreover, in severe human hypertension normalisation of the blood pressure after treatment also normalised the elevated substance P levels.²⁸

The aim of the present study was to investigate if vasodilatation induced by the sensory neuropeptides CGRP and substance P are altered in human hypertension, and relate this to the vasomotor responses of noradrenaline and the smooth muscle depolarisation induced by 60 mM potassium in human subcutaneous arteries and veins.

Study population and methods

Study population

Two main groups of patients (Table 1) were studied, hypertensives ($n = 19$, 13 males/6 females) and normotensives ($n = 17$, 10 males/7 females). The hypertensives were further divided into the subgroups: essential ($n = 13$, 8 males/5 females) and renovascular ($n = 6$, 5 males/1 female) hypertension. The renovascular hypertensive subjects had stenosis of

Table 1 Details of the study population

	Control ($n = 17$)	Essential hypertension ($n = 13$)	Renovascular hypertension ($n = 6$)
Diastolic blood pressure (mm Hg)	80.0 ± 3.3	88.5 ± 5.1	99.2 ± 7.1*
Systolic blood pressure (mm Hg)	147.5 ± 6.6	157.5 ± 3.9	178.3 ± 17.6
BMI (kg/m ²)	23.8 ± 1.0	23.9 ± 1.0	24.2 ± 1.1
Weight (kg)	69.9 ± 2.3	72.0 ± 4.1	74.5 ± 6.0
Creatinin (mmol/L)	96.7 ± 6.4	129.5 ± 14.8	133.8 ± 13.9*
Age (yr)	66.3 ± 3.5	67.0 ± 1.7	67.3 ± 3.1
Smoking (n)	3	9	4

* $P < 0.05$ (Mann–Whitney U-test). n , number of patients.

the arteria renalis diagnosed by a renogram (with capoten scintigraphy) followed by a renal angiography (with pressure recording).

The subjects were recruited from patients undergoing vascular reconstruction mainly for lower limb ischaemia (16 subjects) and vascular abdominal surgery ($n = 6$) and the rest for nonvascular disorders ($n = 14$), mainly inguinal hernia or renal donations. Thus, the vessels were retrieved from the groin or abdominal area.

Creatinine (mmol/L) were similar in the controls and the essential hypertensives, but as expected increased in the renovascular hypertensives. Renovascular hypertensives had elevated diastolic blood pressure. No differences were observed in age, BMI and height (m) between the groups, including the subgroups.

The risk factors were evenly distributed among groups with the exception of smoking which was more abundant in the hypertensives ($n = 13$) versus the normotensives ($n = 4$). Furthermore, the hypertensives received medication for their hypertension (Table 2). Cardiovascular events (myocardial infarction, stroke, transient ischaemic attack (TIA)) were similar in the groups: four in controls, six in hypertensive (six in essential hypertensives and zero in renovascular hypertensives).

Experimental set-up

In accordance with our previously published method description³⁰ cylindrical segments (1–2 mm long) were mounted on two L-shaped metal prongs, one of which was connected to a force displacement transducer (FT03C) attached to a PowerLab unit (ADInstrument, UK) for continuous recording of the isometric tension on a PC computer and the other to a displacement device. The vessel specimens were immersed in temperature controlled (37°C) tissue baths containing a buffer solution of the following composition (mM): NaCl 119, NaHCO₃ 15, MgCl₂

Table 2 Treatment in the hypertensive group

	Essential hypertension	Renovascular hypertension
Ca-channel inhibitors (nifedipine, felodipine, isradipine)	2	5
Alpha-blocker (prazosin)	1	
Beta-blockers (atenolol, metoprolol, betoxalol)	5	4
ACE-inhibitor (ramipril)	3	
Diuretics (thiazide, furosemide, amiloride)	5	3

One subject in the essential hypertensive group had triple treatment, four subjects had double treatment, five had single treatment and two subjects did not receive medication. One subject in the renovascular group had triple treatment, four had double and one had single treatment.

1.2, NaH_2PO_4 1.2, CaCl_2 1.5 and glucose 5.5. The solution was continuously gassed with 5% CO_2 in O_2 giving a pH of 7.4.

The diameter of the vessels internal lumen was measured using a calibrated scale set in the microscope (Olympus SZ60).

A passive tension (arteries 4 mN, veins 2 mN) was applied to segment and they were allowed to stabilise at this level of tension for 1.5 hours. The contractile capacity of each vessel segment was examined by exposure to a potassium-rich (60 mM) buffer solution, which had the same composition as the standard solution except that some of the NaCl was exchanged by an equimolar concentration of KCl.

We used initial potassium induced contractions of each studied vessel segment as a reference. Differences caused by structural changes, general condition and initial tension are thereby largely eliminated. As a precontractor for the vasodilators (CGRP and substance P) we used noradrenaline (10^{-5} M) given as a single dose. Noradrenaline was given after propranolol (10^{-6} M) and cocaine (10^{-7} M) to block the post-junctional dilator effect of the β -adrenoceptor and the neuronal re-uptake, respectively. Each vessel segment was allowed to stabilise before the vasodilator substances were administered in a concentration-cumulative manner. The tone induced by noradrenaline was stable during the period during which the vasodilators were examined.

Statistics

Differences between curves were evaluated by using two-way analysis of variance (ANOVA) by repeated measures, then differences were evaluated using the Kruskal–Wallis test. When single mean values between groups were compared Mann–Whitney U-test was used. All tests were carried out on a Macintosh IIsi using the Statview II software. Results are given as percentage of the noradrenaline-induced contraction for relaxant peptides and as mN for contractile substances. Maximum amount of dilatation/contraction = E_{max} . Potency is expressed as pEC_{50} (–log concentration of agonist inducing half maximum vasodilatation). The data are expressed as mean values \pm s.e.m.

Results

Vessels

Arterial vessel diameter in the total hypertensive group was 0.35 ± 0.06 mm (mean \pm s.e.m.), for essential hypertensives 0.38 ± 0.08 mm and renovascular hypertensives 0.30 ± 0.09 mm. In controls the corresponding value was 0.28 ± 0.04 mm. In veins the diameter for the total hypertensive group was 0.69 ± 0.09 mm and for essential hypertensives 0.63 ± 0.1 mm and for renovascular hypertensives 0.85 ± 0.16 mm. In controls the corresponding value was 0.50 ± 0.1 mm. No differences were shown between the groups.

Potassium

Potassium (60 mM) induced strong contractile responses in all vessel types tested (Table 3). In arteries there was no difference between the hypertensive group (mean \pm s.e.m.) (6.6 ± 1.8 mN) and the controls (4.2 ± 0.6 mN). No differences were shown between the controls and the essential hypertensives (7.3 ± 2.3 mN) or between controls and renovascular hypertensives (5.0 ± 2.9 mN).

In veins there was no difference between the hypertensive group (mean \pm s.e.m.) (6.7 ± 1.7 mN) and the controls (4.2 ± 0.8 mN). Furthermore, there were no differences between the controls and the essential hypertensives (7.5 ± 2.2 mN) or between controls and renovascular hypertensives (5.0 ± 1.7 mN).

Noradrenaline

Noradrenaline 10^{-5} M induced a strong contractile response in all vessels tested. No significant differences in the contractile response between any of the groups in any of the vessel types were demonstrated for this concentration of noradrenaline tested (Table 3). There were no correlation between vessel diameter and contractile response in any of the vessel types tested.

CGRP

The maximum dilatory response in the arteries of the control group was ($E_{\text{max}} \pm$ s.e.m.) $48.6 \pm 8.0\%$ and in the hypertensives $78.7 \pm 5.4\%$ (Table 3, Figure 1). This represents a significant increase ($P < 0.01$) in relaxation to CGRP of the hypertensives. Furthermore, in the essential hypertensives the maximum dilatory response ($77.2 \pm 7.2\%$; Figure 1) was increased ($P < 0.05$) as compared with controls. Corresponding value in the renovascular hypertensive group ($82.0 \pm 7.6\%$; Figure 1) was also increased ($P < 0.05$). The pEC_{50} values did not differ between the controls and hypertensives, or between controls or

Table 3 Contractile vasomotor responses (mean \pm s.e.m.) to noradrenaline and potassium (in mN)

	<i>n</i>	<i>Vessel type</i>	<i>Potassium</i>	<i>Noradrenaline</i>
Control	17	artery	4.2 ± 0.6	7.6 ± 1.9
	8	vein	4.2 ± 0.8	5.5 ± 1.9
Essential hypertension	13	artery	7.3 ± 2.3	5.4 ± 1.1
	10	vein	7.5 ± 2.2	6.5 ± 1.3
Renovascular hypertension	6	artery	5.0 ± 2.9	3.2 ± 0.9
	4	vein	5.0 ± 1.7	5.2 ± 1.1
HT (tot)	19	artery	6.6 ± 1.8	4.7 ± 0.8
	14	vein	6.7 ± 1.7	6.5 ± 1.3

HT (tot), total hypertensive group; *n*, number of patients. No significant differences were observed.

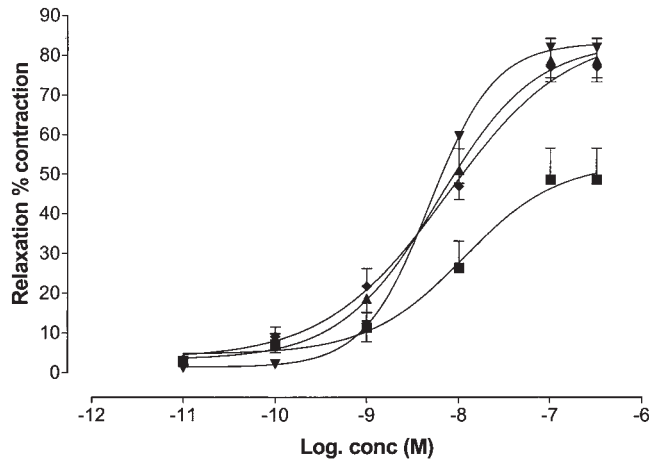


Figure 1 CGRP-induced relaxation in subcutaneous arteries. E_{max} for controls (boxes) differs significantly ($P < 0.01$) from total hypertensives (triangle, top upwards) and from essential hypertensives (diamond, $P < 0.05$) and from renovascular hypertensives (triangle, top downwards, $P < 0.05$).

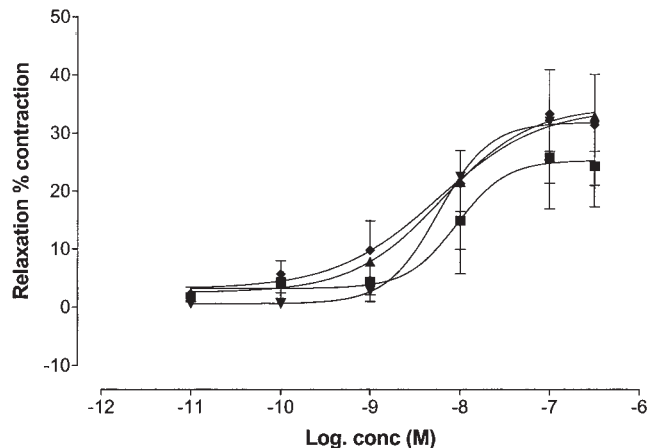


Figure 2 CGRP-induced relaxation in subcutaneous veins. E_{max} for controls (boxes) not different from total hypertensives (triangle, top upwards) or from the subgroups essential (diamonds) or renovascular hypertensives (triangle, top downwards).

essential hypertensives or renovascular hypertensives for this vessel type.

In veins (Figure 2), although generally lower responses were present in the hypertensives ($32.9 \pm 6.1\%$) and the hypertensive subgroups (essential hypertensives: $33.3 \pm 7.7\%$, renovascular hypertensives $32.0 \pm 10.7\%$) as compared with controls ($25.8 \pm 8.9\%$), no significant differences were shown. The pEC_{50} values did not differ between the controls and hypertensives.

There was no correlation between vessel diameter and E_{max} in any of the vessel types tested.

Substance P

The maximum dilatory response in the arteries of the control group was ($E_{max} \pm$ s.e.m.) $55.2 \pm 11.6\%$

Table 4 Vasodilatory responses to CGRP and substance P in human subcutaneous arteries precontracted by noradrenaline

	<i>n</i>	Vessel type	E_{max}	pEC_{50}
<i>CGRP</i>				
Control	17	artery	48.6 ± 8.0	8.09 ± 0.19
	10	vein	25.8 ± 8.9	8.05 ± 0.23
Essential hypertension	13	artery	$77.2 \pm 7.2^*$	8.25 ± 0.15
	10	vein	33.3 ± 7.7	8.50 ± 0.32
Renovascular hypertension	6	artery	$82.0 \pm 7.6^*$	8.31 ± 0.15
	4	vein	32.0 ± 10.7	8.03 ± 0.16
HT (tot)	19	artery	$78.7 \pm 5.4^{**}$	8.27 ± 0.11
	14	vein	32.9 ± 6.1	8.36 ± 0.23
<i>Substance P</i>				
Control	12	artery	55.2 ± 11.6	8.13 ± 0.39
	7	vein	18.8 ± 8.9	8.25 ± 0.72
Essential hypertension	11	artery	41.2 ± 8.3	7.81 ± 0.33
	11	vein	37.7 ± 9.4	8.18 ± 0.32
Renovascular hypertension	6	artery	83.4 ± 8.3	7.53 ± 0.36
	4	vein	34.5 ± 15.5	7.56 ± 0.58
HT (tot)	17	artery	56.1 ± 7.8	7.71 ± 0.24
	15	vein	36.9 ± 7.8	8.00 ± 0.29

HT (tot), total hypertensive group; *n*, number of patients. Values given as means \pm s.e.m. $^{**}P < 0.01$, $^*P < 0.05$.

and in the hypertensives $56.1 \pm 7.8\%$ (Table 4). In the essential hypertensives the maximum dilatory response was ($41.2 \pm 8.3\%$) and in the renovascular hypertensive group ($83.4 \pm 8.3\%$). No significant differences between the hypertensives (total, essential or renovascular) and the controls were shown. However, the renovascular hypertensive group had an enhanced E_{max} as compared to the essential hypertensives ($P < 0.05$). The pEC_{50} values did not differ between the controls and hypertensives, or between controls or essential hypertensives or renovascular hypertensives for this vessel type.

In veins, the maximum dilatory response in the hypertensives was ($36.9 \pm 7.8\%$), and in the essential hypertensives $37.7 \pm 9.4\%$, and in the renovascular hypertensives $34.5 \pm 15.5\%$. No significant differences were shown as compared to controls ($18.8 \pm 8.9\%$). The pEC_{50} values did not differ between the controls and hypertensives.

There were no correlation between vessel diameter and E_{max} in any of the vessel types tested.

Discussion

This study has shown enhanced CGRP-induced vasodilatation in human subcutaneous arteries in hypertension. Furthermore, this increased relaxant response was present in the hypertensive subgroups essential and renovascular hypertension. However, there was no change in pEC_{50} . Substance P-induced vasodilatation was not altered by human hypertension. Furthermore, the contractile responses to potassium and noradrenaline were unaltered in this

study. In veins there was no significant difference for any of the substances tested.

The patients in the study were all non-diabetics, and diabetes may therefore not have influenced the results. The essential hypertensive subjects had moderate hypertension and received standard treatment with good regulation of the blood pressure. Renal failure has not influenced the results since no subject had renal failure. Moreover, the creatinin-levels did not differ significantly between the groups. Cardiovascular events were evenly distributed between the groups. Smoking however, was more frequent in the hypertensives and, hence, this factor may theoretically have influenced the results. Other influences on the results such as long-term alteration induced by the standard medication cannot be ruled out. The renovascular hypertensives did receive medication without reaching proper regulation of their blood pressure and showed elevated diastolic blood pressure. Furthermore, the creatinin levels were increased as compared to controls and this factor may have influenced the results.

We have used potassium (60 mM) to induce contraction of each studied vessel segment as a reference. Differences caused by structural changes, general conditions and initial tension are thereby largely eliminated. Contractile responses to potassium have previously been shown to be unchanged or decreased^{31,32} by human hypertension in subcutaneous arteries. In our study the vasomotor response to potassium was not altered by hypertension in any of the groups, thus indicating no major difference in vascular smooth muscle cells. This is in agreement with the diameter analysis which did not reveal any significant difference between the groups.

Noradrenaline was not added in a cumulative manner in this study, but used as a precontractor in a concentration previously shown to be at the E_{max} level in this kind of human vessel.³³ Studies on noradrenaline-induced vasoconstriction have shown unchanged,^{33,34} increased^{35–37} and decreased³⁸ contractile responses in human hypertension. There were, however, no differences between the groups in response to this dose of noradrenaline in our study. Thus, both contractile agents studied (potassium and noradrenaline) were not altered by human hypertension to the degree studied here. This points toward an unaltered muscle function.

The enhanced CGRP-induced vasorelaxant effect in human hypertension has not been reported before. However, studies in animal models corroborate our results. In SHR rats there is a reduced synthesis of CGRP,³⁹ reduced plasma CGRP level and an enhanced dilatory response.⁸ Furthermore, enhanced CGRP-induced vasodilator responses¹³ and increased depressor responses⁸ have been observed in SHR rats. In DOCA-salt rats there is an increased synthesis of CGRP³⁹ and CGRP has been shown to reverse this hypertension.⁴⁰ In Sprague-Dawley rats with subtotal nephrectomy-induced hypertension⁴¹ and in N_G nitro-L-arginine methyl

ester-induced hypertension in pregnant rats CGRP has been shown to be a potent depressor agent.^{42,43}

The role of CGRP in human hypertension is unclear and the results contradictory. However, there is ample evidence that CGRP may balance the sympathetic nervous system in situations of maximum stimulation or provocation.^{24,44,45} Increased sympathetic nervous system activity occurs in essential hypertension.^{46,47} The result from our study may suggest a physiologic adaptation of the sensory nervous system to the increase in blood pressure and/or increase in sympathetic nervous activity in human hypertension. The mechanism of this putative adaptation would be the attenuation of the blood pressure by a compensatory augmentation of vasodilatory activity. Our results suggest that this augmentation may be present in both subgroups of hypertension. The mechanism involved may be an adaptation to the pressure rather than a phenomenon involved in the pathogenesis of hypertension. However, other mechanisms are possible, eg, reduced expression of CGRP has been observed in animals and could contribute to elevated blood pressure by the relative reduction of vasodilator activity. In this case the enhanced vasodilatory response in hypertension indicate a pathogenic rather than an adaptive role for CGRP in hypertension. Edvinsson *et al*²⁸ have shown a correlation between blood pressure and circulating CGRP levels in severe essential hypertension. This suggests an increased activity in the sensory nervous system as a response to the increased blood pressure and/or increased sympathetic activity. However, the interpretation of such data is difficult since other studies on circulating levels of CGRP in human hypertension show contradictory results.^{9–12} Other common characteristics of hypertension (eg, medication, metabolic disturbances) may also have influenced the results.

In cultured human vascular smooth muscle cells from coronary arteries, mRNA for CRLR and RAMP2 are more abundant than those for RAMP1. But the expression of RAMP1 is dependent of the concentration of corticosterone.⁴⁸ This may suggest a shift in the balance towards production of CGRP receptors rather than adrenomedullin-receptors in steroid dependent hypertension. However, from our study no conclusions can be drawn about the mechanisms behind the enhanced CGRP-induced vasomotor responses in hypertension. More studies are needed in the future to further elucidate this. However, if we apply the operational model of receptor analysis⁴⁹ on our data it would suggest the induction of more CGRP receptors.

In severe human hypertension elevated levels of substance P have been reported,²⁸ although in mild to moderate condition no change could be observed.^{10,24} Furthermore, decreased levels of substance P have also been shown.^{25,26} In severe hypertension, substance P levels returned to normal after treatment of the blood pressure.²⁸ Substance P

levels did not increase concomitantly with the sympathetic neurotransmitter neuropeptide Y during provocation of the sympathetic nervous system (maximum exercise) in healthy volunteers and in hypertensives. In contrast, CGRP levels increased in the same experimental situation,²⁴ suggestive of a compensatory role for CGRP rather than for substance P.

Impaired endothelium-dependent vasodilatation involving substance P in human essential hypertension has been demonstrated.¹⁷ Contradictory, unaltered endothelium-dependent vasodilatory properties in human hypertension have also been shown.^{19,37}

The renovascular hypertensives had an enhanced substance P-induced vasodilatory response compared to the essential hypertensives (but the mechanism behind this could only be speculated on). However, substance P-induced vasodilatation was not altered by human hypertension when compared to the controls in our study.

Since the dilatory effect of substance P is dependent of an intact endothelium this indicates: (a) that the enhanced maximum vascular response to CGRP was not due to an endothelium-dependent mechanism, and (b) the endothelial function is intact in moderate hypertension in this setting. However, the response to endothelium-dependent dilators in hypertension can depend on the agonist used, the pre-contractile agent used and degree of pre-constriction.

Conclusion

CGRP, but not substance P, may induce enhanced vasodilatory responses in small subcutaneous arteries in human essential and renovascular hypertension. This may suggest a physiologic adaptation; attenuation of the elevated blood pressure by a compensatory augmentation of the vasodilatory activity. However, other common characteristics of hypertension (eg, medication, metabolic disturbances) may also have influenced the results.

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