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REVIEW ARTICLE

Nitric oxide and hypertension: not just an endothelium derived relaxing factor!

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The importance of endothelial nitric oxide (NO) generation in sustaining a tonic systemic vasodilatation is well established. Inhibiting NO production produces hypertension in animals and in humans and not surprisingly there has been considerable interest in establishing whether deficiencies of endothelial NO pathway activity are implicated in the aetiology of essential hypertension. The results of these investigations have been inconsistent with some suggestion that observed deficiencies of both basal and stimulated endothelial NO generation in hypertensive subjects may be an effect rather than the cause of raised arterial pressure. It is increasingly recognised that neuronal production of NO also influences cardiovascular homeostasis through its action as a neuromodulator within the autonomic nervous system. Overall NO has been shown to have sympatho-inhibitory and vagotonic effects, acting by both central and peripheral mechanisms. Sympathetic overactivity, coupled with the permissive role of a depressed level of baroreflex mediated cardiac vagal control, may play a significant role in the genesis of human hypertension. Early work in hypertensive rats suggests that neuronal NO production is impaired at a number of key central sites concerned with autonomic cardiovascular regulation. This data is consistent with the pattern of autonomic dysfunction observed in human hypertension. The possibility that neuronal rather than endothelial production of NO might play a significant role in the aetiology of essential hypertension is a promising area for future human research.
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Introduction

The mechanisms initiating primary or essential hypertension remain obscure and it is possible that the term encompasses a group of disorders with diverse aetiologies. The basic determinants of blood pressure are cardiac output and peripheral vascular resistance and the cause or causes of hypertension presumably lie in the myriad factors that control these values. While some young untreated hypertensives demonstrate an increased cardiac output in the initial phase of their disease, in established hypertension an increased peripheral resistance comes to dominate the haemodynamic picture.[1,2] Abnormalities of both vascular structure and function have been proposed to explain this increased vascular resistance. The original hypothesis developed by Folkow[3] postulates that a repeated or sustained functional pressor influence (which may include the relative under activity of a dilator influence) leads to structural changes that in turn reinforce the elevation of peripheral resistance. It is now evident that vascular tone is controlled not only by nervous and hormonal influences but also by locally active factors produced by the endothelium. Arguably the most important these is nitric oxide (NO), whose role as a tonically active vasodilator is now firmly established. Not surprisingly there has been intense interest in the possible role of under activity of the endothelial NO pathway as the causal 'functional pressor influence' leading to establishment of elevated vascular resistance in hypertension.

The autonomic nervous system is another important homeostatic mechanism in the regulation of arterial pressure which also appears to be under the control of nitric oxide. Elevated levels of sympathetic nervous activity may contribute to the development and/or maintenance of hypertension by a number of interacting mechanisms related to effects on the heart, vessels and kidneys (Figure 1). Sympathetic over-activity has been most clearly demonstrated in early hypertension[4–10] but increased muscle sympathetic nerve activity is also present in older patients with established hypertension.[11,12] Excess sympathetic drive is also likely to play an important role in hypertension associated with obesity and the insulin resistance syndrome.[13–15] Impairment of baroreflex mediated vagal responses may also constitute an important permissive factor in the development and maintenance of hypertension. This may be mediated not only by the direct

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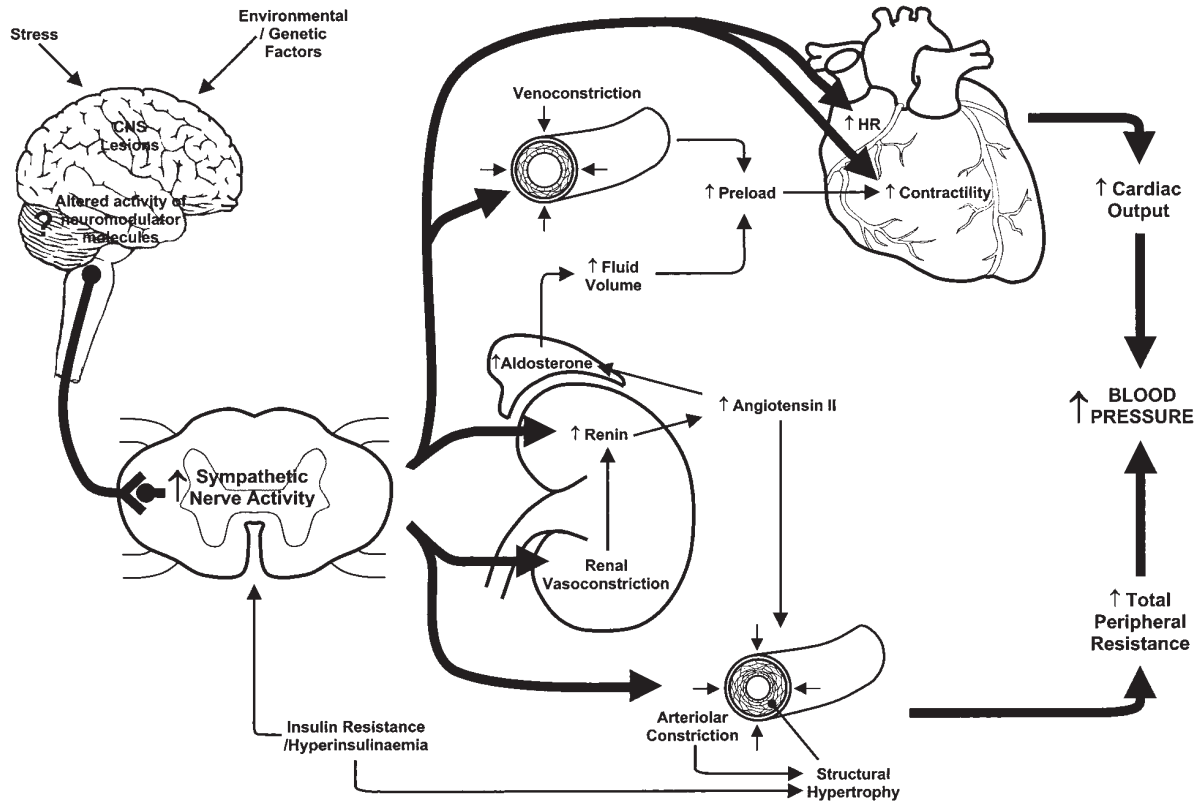

Figure 1 Mechanisms mediating the pressor effect of increased sympathetic activity.

cardio-inhibitory effects of vagal activity but also by its ability to block sympathetic signal transduction, the so-called 'indirect vagal' effect. In the neural pathways of both limbs of the autonomic nervous system there is increasing evidence that neuronally synthesised NO has an important modulatory role. It is possible that abnormalities of the NO pathway within the autonomic nervous system may be equally or more important in the pathophysiology of hypertension than those of the endothelium. This review will examine the role of endothelial and neuronal NO in hypertension and explore the possibility that the neural influences of NO could contribute to the autonomic dysfunction of essential hypertension and possibly to the genesis of the disease itself.

Endothelial NO and hypertension

Nitric oxide is generated from its precursor L-arginine by nitric oxide synthase (NOS). There are three isoforms of the enzyme; the two constitutive forms, endothelial and neuronal NOS (eNOS and nNOS) and the inducible isoform originally described in immune cells (iNOS). Nitric oxide effects its principle biological actions, including that of vascular smooth muscle relaxation, via soluble guanylate cyclase and production of the second messenger c-GMP. The actions of endogenously produced NO can be studied by examining the effects of NOS inhibition using analogues of L-arginine such as N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine (L-NNA) and its methyl ester (L-NAME). Inhibition of NOS results in vasoconstriction and a rise in systemic blood pressure in animals[16–22] and man,[23–27] indicating that resistance vessels are under a tonic vasodilator influence from NO. It is now accepted that the vascular endothelium generates NO via constitutively active eNOS, both under basal conditions and in response to pharmacological (muscarinic agonists) or mechanical stimuli (shear stress related to blood flow). Attenuated vascular NO activity, whether basal or stimulated, might be hypothesised to lead to a relative increase in vascular resistance that is seen in established hypertensives. This is supported by the recent finding that mice with targeted disruption of the eNOS gene are hypertensive.[28]

A number of studies have examined both basal and stimulated vascular NO release in human hypertensive populations. Basal production of vascular NO, assessed by quantification of the vasoconstrictor responses to NOS inhibitors, has been shown to be impaired in human hypertensive subjects.[29,30] Stimulated vascular NO release (endothelial function) has also been shown to be impaired by several investigators. They demonstrated attenuated responses to endothelium depen-

dent vasodilators such as acetylcholine but preserved responses to endothelium independent vasodilators such as sodium nitroprusside in hypertensive patients[31,32] and even in their normotensive offspring.[33] However, not all human trials have confirmed these abnormalities of vascular NO bioactivity in hypertension. Cockroft *et al*[34] studied endothelial function in a larger population of essential hypertensives than previous trials and found vasodilator responses to muscarinic agonists and nitroprusside to be similar to those in normotensive controls. The same group have also recently shown that basal vascular production of NO is preserved in hypertension.[35] These apparent inconsistencies have led to the suggestion that the abnormalities of vascular NO generation/activity found in hypertension may be a result of the increased blood pressure rather than its cause. Not only do the abnormalities of basal NO production correct with normalisation of blood pressure in hypertensive subjects[36,37] but endothelium dependent dilatation is immediately impaired by acute elevation of blood pressure in healthy volunteers.[38]

We therefore lack a clear consensus on whether vascular NO generation is impaired in human hypertension and, if so, whether this represents a cause or effect. It is likely that abnormal NO generation in hypertension is not confined solely to the endothelium. Studies using radio-labelled L-arginine have shown a reduction in total body NO production in human hypertension.[39] The possibility therefore exists that a relative lack of NO activity elsewhere in the body may contribute to the pathogenesis of hypertension.

Neural NO and hypertension

Neuronal NOS has been demonstrated in discrete neuronal populations localised within central nuclei and peripheral autonomic pathways concerned with the regulation of cardiovascular activity.[40] Accumulating evidence now strongly suggests that the NO generated at these sites plays an important role in the regulation of blood pressure acting as a neuromodulator influencing both sympathetic and parasympathetic cardiovascular regulation.

Nitrergic modulation of sympathetic nervous activity

There is substantial evidence to suggest that NO inhibits cardiac and vascular sympathetic activity both centrally and peripherally. A significant component of the hypertensive response to systemic NOS inhibition may be effected not only by removal of the tonic vasodilator influence of endothelial NO generation but also through sympathetically mediated vasoconstriction. Sympathectomised animals show a greatly attenuated hypertensive response to chronic NOS inhibition[41] and the hypertension induced by acute NOS inhibition can be reversed by suppression of central sympathetic outflow.[42] In addition, acute sympathetic ganglion blockade[22,41,43] and acute beta blockade[22] cause a significantly greater fall in blood pressure during chronic NOS inhibition than that seen in control animals suggesting enhanced levels of sympathetic vasoconstrictor activity.

Several investigators have shown an increase in sympathetic nerve activity during systemic NOS inhibition suggesting that endogenous NO causes a tonic inhibition of basal sympathetic activity. In anaesthetised rats, a biphasic response in renal sympathetic nerve activity (RSNA) occurred in response to systemic L-NMMA.[44] Following the initial decrease in RSNA ascribed to baroreflex mediated sympathetic inhibition, there was a sustained increase despite the persistent rise in blood pressure. This increase in activity was abolished by cervical spinal transection suggesting a central action of NO in the inhibition of sympathetic outflow. Pretreatment with L-arginine prevented the late increase in RSNA suggesting that the potentiating effects of L-NMMA on sympathetic activity were due specifically to inhibition of NO synthesis. Other investigators have also shown increases in renal[45] and cardiac sympathetic nerve activity[46] during systemic NOS inhibition when baroreflex activation was prevented. Conversely, in baroreceptor intact rabbits, stimulation of NO synthesis with intravenous L-arginine caused a decrease in cervical sympathetic nerve activity and RSNA despite a fall in blood pressure.[47]

The use of modulators of the NO pathway which lack specificity for a particular isoform of NOS and the systemic route of their administration does not allow us to determine where NO acts to inhibit sympathetic nerve activity. Nor does it determine which of the two constitutively expressed isoforms (eNOS or nNOS) is involved. However, other evidence suggests that both central and peripheral mechanisms come into play, with an important role for nNOS and thus neuronal production of NO.

Central effects on basal sympathetic activity

Direct administration of NOS inhibitors into the brain produces a sympathetically mediated increase in blood pressure and heart rate as well as a rise in directly recorded RSNA.[48–51] These effects are abolished by cervical spinal cord transection.[50] Conversely, stimulation of NOS within the brain by intra-cerebral injection of L-arginine results in a reduction of arterial pressure and directly recorded abdominal sympathetic nerve activity.[52]

There may be several sites at which NO causes inhibition of central sympathetic processing. The nucleus tractus solitarius (NTS) is the primary recipient of afferent baroreceptor fibres entering the medulla and micro-injection of L-NMMA at this site results in an increase in arterial pressure and

RSNA.[53,54] NTS neuronal activity was reduced by systemic injection of L-NAME at constant blood pressure[55] while L-arginine and the NO donor, sodium nitroprusside (SNP) increased discharge rate.[56] The RVLM, the final site of sympathetic processing in the brainstem, also appears to contain neurons susceptible to an inhibitory influence by NO.[54,57,58] Descending inputs to the medullary nuclei from the hypothalamus may also be involved. Studies have shown that NO may be a neuromodulator within the paraventricular nucleus (PVN), a site important for sympathetic regulation in circulatory volume homeostasis. Its effect here is once more to inhibit efferent sympathetic outflow by a mechanism that may be related to GABA.[59,60]

Although there are some contrary results,[42,61–63] a picture emerges of NO acting as a neuromodulator within sites of central sympathetic neuronal integration to effect a tonic inhibition of central sympathetic outflow. This mechanism may be important in limiting sympathetic activation during stress as it has been observed that the number of NO producing neurons at key sites of autonomic processing in the brains of conscious rats increases in response to experimentally induced stress.[64]

Some *in vivo* evidence does exist to suggest that endogenous NO also results in tonic inhibition of efferent sympathetic nerve activity in humans. The systemic infusion of L-NMMA to healthy volunteers at a dose designed to minimise baroreflex loading resulted in higher levels of directly recorded muscle sympathetic nerve activity than an equipressor doses of phenylephrine, given as a non-NO dependent control vasoconstrictor.[26,27]

Central effects on reflex sympathetic nerve activity

The effect of NO on the baroreflex control of sympathetic activity remains unclear. Some studies have suggested that NO inhibits baroreceptor-RSNA gain, as evidenced by an increase in reflex gain during acute systemic NOS inhibition.[45,49,61] However, a number of studies have failed to show any influence of NO on the reflex control of sympathetic activity.[47,62] This inconsistency is only partially explained by species differences or the effect of anaesthetics.

More complex dynamic indices of baroreflex sympathetic control may be influenced by NO. If sustained pressure is applied to the vascularly isolated rabbit carotid sinus, rapid inhibition of RSNA followed by a gradual return towards baseline occurs, a process known as 'rapid central adaptation'. The magnitude and rate at which this adaptation occurred was increased by intracisternal L-NAME.[51] This suggests that endogenous NO may act within the brainstem to reduce central adaptation, and thus sustain the reflex inhibition of sympathetic activity during a rise in arterial pressure.

Modulation of cardiac and vascular responses to sympathetic stimulation

Cardiac tissue: In addition to controlling levels of efferent sympathetic activity there is evidence that NO may also attenuate the end-organ response to sympathetic stimulation. In experiments on cultured rat myocytes, inhibition of the NO signal transduction system caused myocytes to show enhanced inotropic responses to beta-adrenergic stimulation.[65] Conversely, exogenous NO donation with SNP attenuated both the chronotropic and inotropic responses to sympathetic nerve stimulation in isolated guinea-pig atria,[66] as well as the inotropic response to isoprenaline in human atrial and ventricular muscle strips.[67] This effect has also been confirmed *in vivo* with a significant enhancement by L-NAME of the inotropic effect of intracoronary dobutamine in dogs.[68]

Cardiac myocytes constitutively express eNOS and work with cultured cells would suggest that it is this isoform that generates the NO responsible for the inhibition of cardiac sympathetic responsiveness.[65] However the demonstration of increased heart rate responses to sympathetic nerve stimulation after the administration of selective nNOS inhibitors in the vagotomised rabbit[69] and ferret[70] indicate that neurally produced NO may also contribute significantly to this effect *in vivo*. This may be due to pre-synaptic inhibition of noradrenaline release from cardiac sympathetic nerves.[71,72] The recent description of nNOS in the cardiac sarcoplasmic reticulum[73] may mean that this isoform also contributes to a post-synaptic inhibition of adrenergic signal transduction possibly by modulating intracellular calcium currents.[74–76]

Not all data on the cardiac effects of NO have been consistent. Recently some confusion has arisen from attempts to ratify the results of experiments using NOS inhibitors with those of exogenous NO donors. Although not shown in all species,[77] the modulation of beta-adrenergic cardiac responses by exogenous NO seems to display a concentration dependent biphasic profile. Low concentrations of NO donors increase contractile responses to electrical nerve stimulation, whereas higher doses produce a negative inotropic effect.[78,79] The inotropic effect of NO also appears to depend on the level of background sympathetic activity since the transition from positive to negative inotropy is shifted towards a lower dose of NO donor—and possibly levels of cGMP production that are more representative of normal physiology—by a beta-adrenergic agonist.[79] This ties in well with the findings of the NOS inhibitor experiments reviewed above, which suggest that the action of endogenous NO generation is to inhibit, rather than potentiate, inotropic responses to beta-adrenergic stimulation. Finally, this paradigm is also supported by findings that young eNOS knockout mice (prior to the development of ventricular hypertrophy) also display enhanced inotropic

responses to beta-adrenergic agonists (for review see Balligand[80]).

Vascular tissue: Modulation of central sympathetic activity cannot explain vasoconstrictor responses to localised intra-arterial administration of NOS inhibitors in human experiments, with the removal of the direct vasodilator influence of endothelial NO believed to be solely responsible.[16] However, peripheral neurogenic mechanisms could also contribute to this effect since NO may attenuate the vasoconstrictor response to basal sympathetic nerve activity. *In vitro*, NOS inhibition enhanced vasoconstrictor responses to both noradrenaline and sympathetic nerve stimulation,[81] whilst NO resulted in attenuation of the constrictor effects of sympathetic nerve stimulation.[82] These actions may reflect a non-specific dilator influence of NO, however a specific pre-synaptic inhibitory influence of NO on sympathetic vasoconstriction has been demonstrated. Exogenous NO reduced the efflux of radiolabelled noradrenaline in response to nerve stimulation of canine pulmonary vessels[83] and mesenteric artery.[84] Also a specific post-junctional interaction between NO and noradrenaline was suggested by the finding that L-NAME enhanced vasoconstrictor responses to noradrenaline much more than responses to angiotensin II.[42]

Nitrergic modulation of vagal cardiac control

Impairment of cardiac vagal action in hypertension may be an important mechanism underlying the abnormalities of baroreflex function seen in established hypertension. Additionally, cardiac vagal innervation represents an important sympatho-inhibitory mechanism. There is good evidence that NO increases activity in brainstem sites that promote efferent vagal activity, and also enhances cardiac responses to vagal stimulation.

The stimulation by NO of neuronal activity within the NTS discussed above provides a possible mechanism by which central vagal activity might be increased by NO as the NTS provides excitatory inputs to vagal motonuclei in the medulla. Further evidence of a central vagotonic effect of NO is provided by recordings of motoneuron activity within the dorsal motor nucleus of the vagus (DMV). Here direct application of NO donors and L-arginine increased firing rate whilst application of a NOS inhibitor decreased firing rate.[85]

An important role for NO in the peripheral control of vagal activity is also evident. The bradycardic response to muscarinic stimulation *in vitro* was partially blocked by inhibitors of the NO-cGMP pathway.[65] In the ferret, inhibition of NOS activity by L-NMMA attenuated the bradycardic response to efferent vagus nerve stimulation, an effect that was reversed by L-arginine.[86,87] Notably this effect was reproduced by a specific nNOS inhibitor.[88] Conversely, NO donors significantly enhanced the bradycardic response to vagal stimulation, in rabbits.[89]

Aside from its direct cardio-inhibitory effects the vagus also exerts a powerful regulatory influence through inhibition of beta-adrenergic responses; this has been termed 'indirect' cardiac vagal activity or 'accentuated antagonism'. Muscarinic receptor stimulation was thought to effect this interaction largely due to inhibition of adenyl cyclase via an inhibitory G protein. However, there is now good evidence that this action may also be mediated by the NO-cGMP pathway. *In vitro*, inhibition of the NO-cGMP pathway has been shown to significantly reduce muscarinic attenuation of adrenergic increases in both contractility[90] and heart rate.[91] *In vivo*, intracoronary L-NMMA attenuated the inhibitory action of vagal stimulation on the inotropic responses to dobutamine in closed chest dogs.[92]

Nitric oxide also appears to have a cardiac vagotonic influence in humans. In a recent study in healthy human subjects, heart rate variability was used to study the modulatory influence of NO on cardiac vagal control. It was observed that although L-NMMA and phenylephrine (administered as a non-NO-dependent control vasoconstrictor) caused equal rises in blood pressure, there was significantly less baroreflex mediated increase in cardiac vagal activity with the NOS inhibitor than with phenylephrine. Conversely, during hypotension resulting from the NO donor sodium nitroprusside, there was relative preservation of indices of vagal activity compared to those produced by an equal fall in blood pressure with the non-NO dependent vasodilator, hydralazine.[93]

Activity of the neural NO pathway in hypertension

The accumulating data strongly suggest a significant role for NO as a neuromodulator of cardiovascular autonomic activity in normal physiology. In pathological states such as hypertension there may be abnormalities of neuronal NO pathway activity at important cardiovascular regulatory sites. Studies of localised 3H-citrulline formation, a by-product of NOS in the formation of NO from L-arginine, have suggested that nNOS activity is reduced in the dorsal brainstem of spontaneously hypertensive rats.[94] Furthermore, intracerebroventricular (ICV) injection of inhibitors of NOS and c-GMP resulted in a smaller pressor response in spontaneously hypertensive rats than in normotensive control animals. Similarly, stimulating brain NOS by ICV injection of calcium (a required cofactor of constitutive NOS) resulted in an attenuated blood pressure response in the hypertensive animals. The fall in arterial pressure seen with ICV administration of NO in hypertensive animals was not only preserved but was significantly enhanced.[95] This evidence suggests that genetically hypertensive rats have reduced levels of basal and

stimulated functional nNOS activity in central sites concerned with the autonomic regulation of blood pressure.

Experimental models of secondary hypertension (eg, renal-clip and mineralocorticoid induced) have also shown reduced expression of nNOS mRNA in sites concerned with central sympathetic regulation such as the hypothalamus[96,97] and the RVLM.[97] Hypothalamic nuclei such as the paraventricular nucleus (PVN) are particularly concerned with the autonomic regulation of circulating volume and abnormalities of nNOS gene expression in these areas may be linked to salt-sensitive hypertension. In normotensive rats, salt loading enhances nNOS gene expression in the PVN and other hypothalamic nuclei. Rats with salt-sensitive hypertension have decreased hypothalamic nNOS mRNA expression.[98] It may be postulated that this may limit their ability to reduce sympathetic outflow to the cardiovascular system and the kidney during salt loading. A further site of action of nNOS in the prevention of salt-sensitive hypertension may within the kidney itself. The enzyme is highly expressed in numerous sections of tubular epithelium and in particular in the macula densa where it may play a role in the regulation of renal sodium handling. Nitric oxide generation in the kidney appears to promote sodium excretion, a role that is in keeping with observations that Dahl salt-sensitive rats made hypertensive by a high salt diet exhibit impaired renal nNOS activity (for review see Kone and Baylis[99]).

If, as the evidence suggests, neural NO is a sympatholytic and vagotonic agent then the reduced levels of this neuromodulator seen in brains of hypertensive animals may bear a causal relationship, or at least be a significant contributor to, the sympathetic over activity and vagal withdrawal observed in this disease state. The role of neuronal NO in the aetiology of hypertension does not seem to be supported by data from nNOS knockout mice who, unlike their eNOS knockout counterparts, do not develop hypertension.[100] However, negative findings such as these in gene knockout animal models must be interpreted with caution. These animals have been deficient in the relevant enzyme throughout embryonic and post-natal development leading to both structural adaptations and the development of compensatory pathways, hence it cannot be inferred that no role is played by the relevant enzyme in the intact animal. It is possible for example that nNOS knockout mice may not suffer hypertension due to compensatory increases in eNOS. Furthermore, although these animals display impairment of basal cardiac parasympathetic activity, the observation that they do not show evidence of increased sympathetic activity may also help to explain the lack of ensuing hypertension.[100] Presumably, this is also the result of compensatory adaptive mechanisms as it is at odds with the findings of central pharmacological NOS inhibition[50]).

Conclusions

Impairment of endothelial NO activity has been documented in primary hypertension. Expectations that this might be a key factor in the increased vascular tone that characterises established hypertension have been marred by evidence suggesting that impaired endothelial NO production appears to be a secondary effect rather than the primary cause of elevated arterial pressure. Animal and human evidence shows that neurally produced NO can also regulate haemodynamic control through modulation of cardiovascular autonomic activity. Central and peripheral effects on both sympathetic and vagal control have been demonstrated.

There is preliminary evidence to suggest that neuronal NO production may be reduced in hypertension, and this finding is certainly consistent with the observed pattern of sympathetic over activity and vagal impairment. Sympathetic hyperactivity in particular may represent an important trigger in the cascade of compensatory mechanisms which eventually lead to established hypertension. Thus the possible role of NO in the genesis of hypertension might not be confined merely to its direct actions on vascular tone but may also involve powerful modulatory effects on cardiovascular autonomic control. Our knowledge of the defects of neural NO pathways in hypertension is currently confined to rat models and further research in this area is required. In particular the role of neronal NO in human hypertension remains unaddressed. The use of specific inhibitors of nNOS in humans could allow future examination of this important question.

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