Evaluation of the involvement of nitric oxide and substance P in reducing baroreflex gain in the genetically hypertensive (GH) rat

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The attenuation of baroreflex gain associated with hereditary hypertension could involve abnormal signalling by nitric oxide or substance P. Baroreflex gain was measured in age-matched male genetically hypertensive (GH) and normotensive (N) anaesthetised rats from heart rate changes in response to i.v. phenylephrine or sodium nitroprusside. In subgroups of these animals, nitric oxide synthesis was inhibited using NG-nitro-L-arginine methyl ester (L-NAME, 30 mg·kg^{-1} i.v.), substance P transmission was blocked using the antagonist SR 140333 (360 nmoles·kg^{-1} i.v.) or substance P release was inhibited with resiniferatoxin (4 doses of 0.3 μg·kg^{-1} i.v. at 4 min intervals). Baroreflex gain was markedly reduced in GH compared to N animals (N –0.37±0.04 beat·min^{-1}·mm Hg^{-1}, GH –0.17±0.02 beat·min^{-1}·mm Hg^{-1}, p<0.0001). Inhibition of nitric oxide synthase increased baroreflex gain in each strain, but the inter-strain difference in gain persisted (post-treatment N –0.57±0.07 beat·min^{-1}·mm Hg^{-1}, GH –0.24±0.05 beat·min^{-1}·mm Hg^{-1} (p<0.001). Blockade of receptors or inhibition of substance P release did not affect gain in either strain.

Nitric oxide, but not substance P, appears to play an inhibitory role in the rat arterial baroreflex. Impairment of baroreflex gain in GH rats is not secondary to altered nitric oxide signalling.

Keywords: genetically hypertensive rat, baroreflex, nitric oxide, substance P, resiniferatoxin

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The majority of animal studies of arterial hypertension utilise one or other of the inbred strains of Wistar rat that exhibit inherited hypertension. One of the two most commonly used strains is the spontaneously hypertensive rat (SHR) that originated from Japan (24). The other is the genetically hypertensive (GH) strain developed in New Zealand (27). Back-cross experiments have shown that the hypertensive trait in the SHR is carried by only a small number of genes while that in the GH involves many genes (24, 27, 35), suggesting that the GH strain may be a more appropriate model of human essential hypertension, which is similarly polygenic (14). It is therefore important to document the functional characteristics of the GH, for comparison with those of the SHR.

Chronic hypertension is known to alter signalling of the arterial baroreflex through the nucleus tractus solitarius (NTS) (10, 19, 23), resulting in a pronounced depression of baroreflex gain. The synaptic changes that underlie this depression are uncertain. However, recent studies have found evidence for involvement of endogenous nitric oxide (NO) in regulation of baroreflex function (30, 31) and the same workers showed depression of this signalling in the SHR. As well, several lines of evidence have suggested that endogenous substance P (SP) within the NTS modulates baroreflex gain (4, 11, 20), although no assessment of this modulation has been made in genetically hypertensive animals. In the present study, we have used inhibition of NO synthase, antagonism at the NK_1 receptor or depletion of SP with resiniferatoxin in anaesthetised animals to assess the roles of endogenous NO and SP in attenuating arterial baroreflex gain in the GH rat.

Methods

The rats used were age-matched adult males (16–20 weeks) bred in the Trinity College Bioresources Unit from stock obtained from the Wellcome Medical Research Institute, University of Otago (New Zealand) and consisting of GH and control animals from the colony that produced the original GH strain (27). The integrity of each line was confirmed by establishing that the systolic arterial blood pressures of adults of each generation as measured by tail-cuff plethysmography were consistently <145 mm Hg in control animals and >190 mm Hg in GH animals.The experimental protocols were approved in advance by the local ethics committee.

Animals were anaesthetised with urethane (1.25 mg·kg^{-1} i.p.) and placed in a supine position on an operating table maintained at 34 °C. The right femoral artery was cannulated for recording of arterial blood pressure and a catheter was placed in the right external jugular vein for infusion of drugs. Taking care to not damage the vagus or sympathetic nerve trunks, loose threads were passed around each common carotid artery

in order to be able to verify baroreflex integrity at the beginning of each experiment by bilateral carotid occlusion. A conventional three-lead ECG was recorded using transcutaneous needle electrodes and used to trigger a ratemeter for monitoring heart rate. All measured parameters were displayed and stored using a PowerLab system (AD Instruments).

Stepwise changes in arterial pressure by 20–100 mmHg were induced by slow (not more than 0.2 ml·min^{-1}) intravenous infusions of phenylephrine (25–$200 \text{ nmoles·min}^{-1}$) and sodium nitroprusside (7–$60 \text{ nmoles·min}^{-1}$). Heart rate responses to the maximum pressor and depressor effects evoked in each animal were measured and baroreflex gain was expressed in terms of the incremental change in heart rate (beat·min^{-1}) per mmHg change in blood pressure. In some animals, NO synthase (NOS) was inhibited using N^G-nitro-L-arginine methyl ester (L-NAME, 30 mg·kg^{-1} i.v., 30 min before assessing baroreflex gain). In others, involvement of intracerebral neurokinin-1 (NK_1) receptors in the baroreflex was assessed by administration of the NK_1 antagonist SR 140333 ($360 \text{ nmoles·kg}^{-1}$ i.v., 30 min before) and the effect of blocking synaptic SP release was assessed by administration of the vanilloid receptor agonist resiniferatoxin (4 doses of 0.3 μg·kg^{-1} i.v. at 4 min intervals). Effects on the CNS of these compounds administered systemically have previously been demonstrated: L-NAME (5, 13); SR 140333 (16); resiniferatoxin (3).

All data were expressed as means $\pm$ S.E.M. and statistical significance of differences between means was calculated using Welch's t-test (InStat, GraphPad Software).

Results

Resting values

For all animals used, resting mean arterial pressures under anaesthesia were 108 ± 5 mmHg in N animals and 158 ± 3 mm Hg in GH animals ($p<0.001$, n = 22 each strain). Resting heart rates were similar between strains (N $385\pm12 \text{ beat·min}^{-1}$, GH $378\pm6 \text{ beat·min}^{-1}$).

Responses to vasoactive drugs

Both strains responded to the same range of concentrations of phenylephrine and showed approximately similar maximal pressor responses (N 99 ± 4 mm Hg, GH 127 ± 7 mm Hg). However, the GH animals exhibited substantially greater responsiveness at lower concentrations of phenylephrine, with an approximately 2-fold elevation in

pressor effects of concentrations below 100 nmoles·min^{-1} ($p<0.001$). Both strains showed similar depressor responses to the lowest concentration of nitroprusside infused (N 36±3, GH 41±5 mm Hg) but all higher concentrations produced greater effects in GH than in N, with responses at the highest concentration being 65±4 and 84±5 mm Hg respectively ($p<0.001$).

Baroreflex gain

Initial bilateral carotid occlusion produced substantial pressure elevation and tachycardia in all animals. Absolute pressor responses were significantly greater in N than in GH animals (N 67±7 mm Hg, GH 39±4 mm Hg, p<0.001) and heart rate responses showed a non-significant trend in the same direction (N 31±3 beat·min^{-1}, GH 24±3 beat·min^{-1}, $p=0.08$).

Baroreflex-mediated heart rate changes were similar in amplitude during pressor and during depressor challenges in both N and GH animals and tachycardic and bradycardic data were therefore pooled for group comparisons. For all N animals tested (n=21), the baroreflex regression equation was $y = -0.37x+2.69$. The slope of the regression line was taken as the mean baroreflex gain and was calculated as -0.37 ± 0.04 beat·min^{-1}·mm Hg^{-1} (Fig. 1a). By contrast, baroreflex gain was significantly lower in GH animals ($p<0.001$, n=21), with a regression equation of $y=-0.17x+0.50$ and a calculated mean gain of -0.17 ± 0.02 beat·min^{-1}·mm Hg^{-1} (Fig. 1b).

Effects of NOS inhibition

In N animals (n=7), treatment with L-NAME approximately doubled baroreflex gain, from -0.27 ± 0.04 beat·min^{-1}·mm Hg^{-1} to -0.57 ± 0.07 beat·min^{-1}·mm Hg^{-1} ($p<0.01$). A similar degree of increase was seen in GH animals n=7), but the absolute gain post-L-NAME in GH remained around one-half of that in the N group (before -0.12 ± 0.02 beat·min^{-1}·mm Hg^{-1}, after -0.24 ± 0.05 beat·min^{-1}·mm Hg^{-1}) (Fig. 2).

L-NAME also caused substantial elevation of resting blood pressure in both N and GH, by approximately 54% in each strain, with concomitant bradycardia (N before 111±8 mm Hg, 399±8 beat·min^{-1}, after 172±12 mm Hg, 359±10 beat·min^{-1}; GH before 163±5 mm Hg, 387±5 beat·min^{-1}, after 254±5 mm Hg, 323±7 beat·min^{-1} (p<0.001 blood pressures, p<0.01 heart rates)).
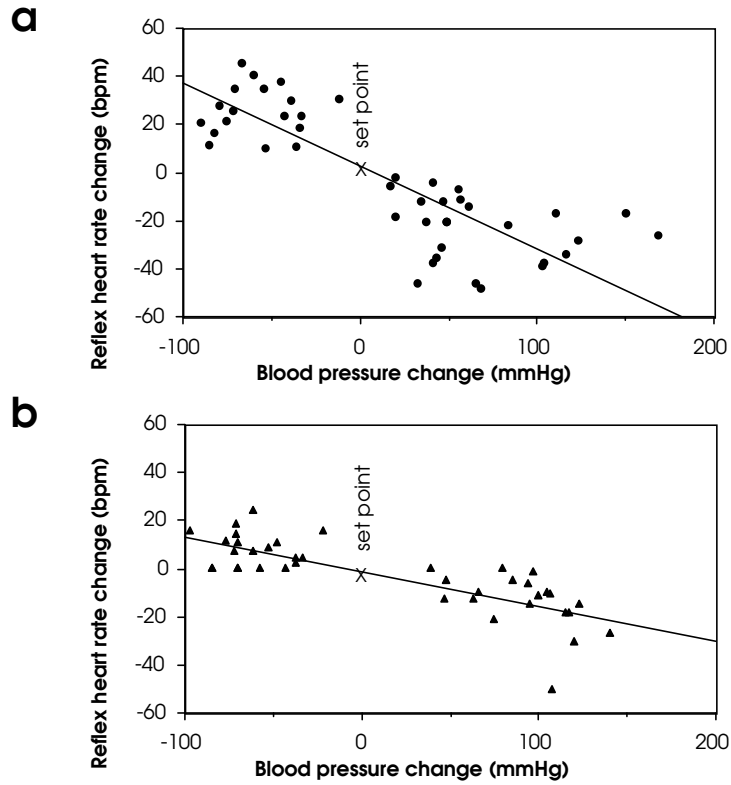
Fig. 1. Linear regression plots of baroreflex-mediated changes in heart rate in response to step-wise changes in blood pressure produced by intravenous infusions of phenylephrine and sodium nitroprusside in (a) normotensive (N) and (b) genetically hypertensive (GH) rats. The slopes of these plots in subsets of the two populations represent the resting baroreflex gain values in the succeeding figures

Effects of NK$_1$ receptor blockade

Pre-treatment with SR 140333 had no effect on resting blood pressure or heart rate in either strain. In addition, there was no effect on baroreflex gain in either N (before –0.44±0.06 beat·min^{-1}·mm Hg^{-1}, after –0.51±0.08 beat·min^{-1}·mm Hg^{-1}, n=7) or GH (before –0.19±0.03 beat·min^{-1}·mm Hg^{-1}, after –0.21±0.03 beat·min^{-1}·mm Hg^{-1}, n=7) (Fig. 3a).

Effects of resiniferatoxin

Resiniferatoxin had no consistent effect on blood pressures, but caused a significant increase in resting heart rate of 20–30 beat·min^{-1} in both strains. No effect on baroreflex gain was observed in either N (before –00.39± 0.09 beat·min^{-1}·mm Hg^{-1}, after –0.41±0.06 beat·min^{-1}·mm Hg^{-1}, n=7) or GH (before –0.19±0.04 beat·min^{-1}·mm Hg^{-1}, after –0.10±0.03 beat·min^{-1}·mm Hg^{-1}, n=7) (Fig. 3b).

Discussion

Chronic elevation of blood pressure has been noted as being associated with resetting of baroreflex gain to a lower value both in a variety of animal models (1, 10) and in man (33). This effect is not secondary to structural changes in the arterial wall since it can be produced and reversed by quite brief periods of blood pressure change (22, 40), indicating that it is due to a functional change in gain of the neuronal reflex circuitry. In agreement with these background data, we found that genetically hypertensive rats from the inbred GH strain exhibit considerably reduced baroreflex gain, approximately half of that exhibited by age-matched normotensive controls from a similar genetic background.
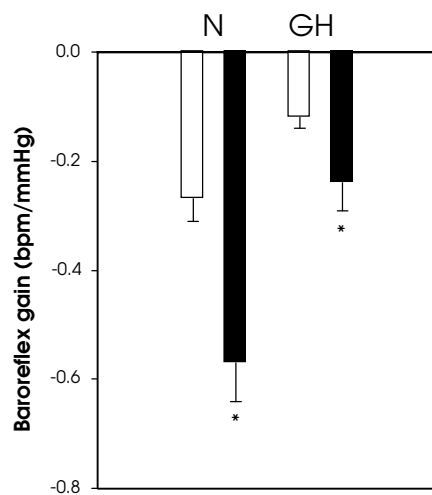

Fig. 2. Baroreflex gain (beats·min^{-1}·mm Hg) in normotensive (N) and genetically hypertensive (GH) rats under control conditions (open columns) and following systemic administration of L-NAME (black columns). Asterisks denote significant effects of L-NAME treatment within groups. Baroreflex gains were significantly greater in N than in GH animals both before and after treatment (n=7 each group)
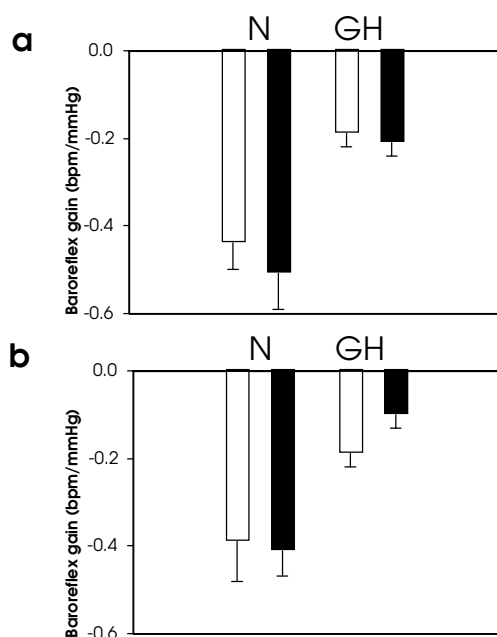
Fig. 3. Baroreflex gain (beats·min^{-1}·mm Hg) in normotensive (N) and genetically hypertensive (GH) rats under control conditions (open columns) and following systemic administration of (a) the NK$_1$ receptor antagonist SR 140333 (black columns) or (b) resiniferatoxin (hatched columns). Responses were significantly greater in N than in GH animals both before and after treatment and neither treatment significantly affected responses in either group (n=7 each group)

The enzyme activity catalysing the formation of NO, NOS, is known to be present in some neurons in the area of the rat NTS (15, 21, 29, 30, 32) and administration of inhibitors of NO synthesis has suggested that endogenous NO may play a functional role in the arterial baroreflex in this species, although the nature of this effect is controversial. Thus, some studies have reported that blood pressure is elevated by intra-NTS delivery of NOS inhibitors (36, 37), while other groups have reported no effect (30). Similarly, Qadri et al. (31) found that chronic inhibition of NOS reduced baroreflex gain, while Paton et al. (25) found evidence in acute experiments for release of NO itself depressing gain. It is possible that some of this variability in results is due to the different effects on reflex activity of NO produced in the NTS by nNOS and iNOS. Thus, Chan et al. (12) produced evidence that the NO generated by nNOS results in sympatho-excitation, while that produced by iNOS causes sympatho-inhibition.

In the present study, we found that acute administration of the NOS inhibitor, L-NAME, produced an approximate doubling of baroreflex gain. Thus our data are in

agreement with the findings of Paton et al. (25) and with the concept that endogenous NO acts as an inhibitory modulator of the baroreflex. This does not, however, necessarily imply that the action is a synaptic one. L-NAME is a non-selective NOS inhibitor with, if anything, a greater effect on eNOS than on nNOS, and Paton et al. (25) have produced several lines of evidence to suggest that the eNOS isoform of the enzyme is the one associated with baroreflex modulation, raising the possibility that transduction of information between vasculature and neurons is involved. As well, peripheral eNOS inhibition could stiffen the carotid arterial wall with consequent elevation of reflex gain.

We found that L-NAME treatment increased baroreflex gain in GH animals to a similar degree as occurred in the N strain, but that, following NOS inhibition, there was still a two-fold proportional difference in gain between GH and N. These findings indicate therefore that, at least in this type of hypertension, the resetting of baroreflex gain cannot be attributed to an abnormality in central nitrergic signalling. This finding is of particular interest because of the contrast it provides with available data on the SHR. Here, it has been reported that brain stem NOS activity is lower than in the normotensive WKY control strain (30) and that baroreflex gain is affected less than is that of WKY by inhibition of NOS (30, 31).

Studies of isolated tissue preparations in both SHR and GH have indicated reduced endothelial dilator function relative to that in the respective normotensive control strains (2, 18, 39) and studies in hypertensive humans similarly suggest a decreased capacity for endothelial release of NO (7, 8). However, acute inhibition of eNOS *in vivo* has been reported to elevate blood pressure to a greater extent in SHR than in WKY (2, 18), compatible with greater endothelium-dependent vasodilation at rest in the hypertensive animals. By contrast, we found that acute L-NAME treatment resulted in a closely similar elevation of resting blood pressures in N and GH strains, indicating that endothelium-derived dilator function is unchanged from normal in the intact GH. Tucker et al. (38) observed with chronic NOS inhibition that blood pressure rose in GH but did not change significantly in N animals. The reason for the difference between those results and our own is not clear. It may however relate to different degrees of inhibition of different NOS isoforms under different experimental circumstances. Tucker et al. (38) proposed on the basis of their observations that defective endothelial NO production might underlie the genesis of hypertension in the GH. While our data do not exclude such a possibility, they provide no support for it.

The hindbrain pathways associated with regulation of arterial blood pressure in the rat may be subject to inhibitory modulation not only by NO but also by the neuropeptide SP. Locally applied exogenous SP has been well-documented to affect blood pressure and heart rate, but the relationship of this to baroreflex function has not been clear. Thus, some workers have reported that exogenous SP enhances reflex gain (4, 9, 11, 20) while others have found no effect on gain (6, 17). Similarly,

administration of NK_1 receptor antagonists has been reported variously to depress (9) and to not affect (26) baroreflex gain. Because the effects within the NTS of SP on cardiovascular sympathetic drive appear to be mediated by NK_1 receptors (17), we assessed the effect on baroreflex gain of the highly selective NK_1 antagonist, SR 140333 (16). We found no effect of blocking these receptors either on resting cardiovascular status or on baroreflex gain, in N or GH animals. In order to target another component of any putative SP-mediated signalling within the baroreflex pathway, we administered the vanilloid receptor agonist resiniferatoxin (34) and found that this, also, had no effect on baroreflex gain in either strain.

In conclusion, our results support a role for endogenous NO in inhibitory modulation of arterial baroreflex gain in the rat, but, in contrast to results in the SHR, do not indicate any abnormalities of either hind brain or endothelial NO production in the GH strain of hypertensive rat. Further, we find no evidence for the participation of endogenous SP in modulating baroreflex sensitivity in either normotensive or GH strains.

Acknowledgements

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