Impaired cardiac vagal activation and down regulation of guanylate cyclase in the spontaneously hypertensive rat

Susanna C. Almond and David J. Paterson

University Laboratory of Physiology, Parks Road, Oxford. OX1 3PT

Introduction

Nitric oxide (NO) is an important signalling molecule in the regulation of vascular resistance and has been implicated in the aetiology of hypertension.

Hypertension is associated with decreased vagal tone, in patients with established hypertension[1], and in animal models of hypertension such as the spontaneously hypertensive rat (SHR)[2].

Hypertension is also associated with down regulation of guanylate cyclase (GC) in the aorta from SHRs, and this precedes the onset of hypertension[3].

In the heart, the NO/ guanylate cyclase/ cyclic GMP dependent pathway enhances the negative chronotropic effect of vagal nerve stimulation[4]. In addition, NO also facilitates the release of acetylcholine (ACh) by a pre-synaptic mechanism.

Aim of the study

The aim of this study was to establish whether the cholinergic regulation of heart rate is attenuated in hypertensive rats (SHRs) compared to normotensive (WKY) controls, and to establish if this is associated with reduced expression of guanylate cyclase.

Methods

320-410g male SHRs (n=20) and WKYs (n=26), 16-20 weeks old were used for the study.

A double atrial / right vagal preparation was dissected free, placed in an organ bath containing oxygenated Tyrode's solution (37ºC) and connected to an isometric force transducer.

Heart rate was triggered from contraction. The change in heart rate with vagal nerve stimulation for 30s or bath applied carbamylcholine (CCh, 100-500nM) was measured. Drugs were added to the preparation after control protocols were completed.

Tissue samples (left ventricle, right atrium and aorta) from SHR and WKY rats were used for Western blot analysis. The primary antibody was polyclonal rabbit anti-sGC α1- and β1-subunit.

Protocols

1. The heart rate response to vagal nerve stimulation (3Hz, 5Hz, 7Hz, 10Hz; 10-15V, 1-2ms duration for 25 seconds at 1 minute intervals) in SHR was compared with WKY rats. The order of stimulations was randomised.

2. The heart rate response to bath applied CCh (100, 200, 500nmol/L) in SHR was compared with WKY rats.

3. The effect of the NO donor, SNP (20mmol/L), on the HR response to vagal nerve stimulation (at 5 and 10Hz) was compared in SHR and WKY rats.

There was no significant difference in baseline heart rate (WKY, average = 224±7 bpm; SHR, average = 245±5 bpm).

Results

Figure 1A

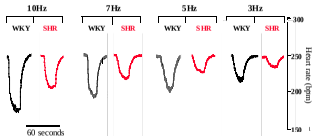


Figure 1B

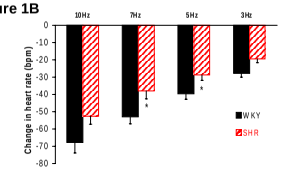


Figure 1A. Raw data trace showing the effect of vagal nerve stimulation at 10Hz, 7Hz, 5Hz and 3Hz on heart rate in isolated rat atrial preparations from a SHR and a WKY rat.
Figure 1B. The heart rate response was significantly smaller in SHR compared to WKY rats ($p<0.05$,WKY n=7, SHR n=9) at 7Hz, 5Hz and 3Hz and at 10Hz, p=0.07.

Figure 2A

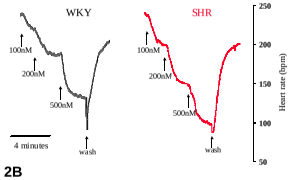


Figure 2B

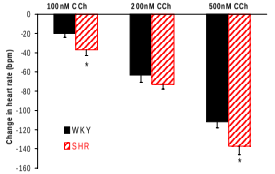


Figure 2A. Raw data trace showing the heart rate response to bath applied CCh (100, 200 and 500nmol/L) added cumulatively in a SHR and WKY rat atrial preparation.
Figure 2B. There was a small significant increase in the heart rate response to bath applied CCh at 100 (n=6) and 500nmol/L (n=12) ($p<0.05$).

Figure 3A

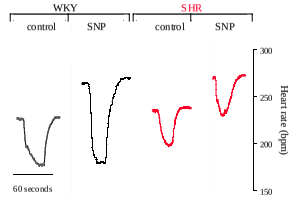


Figure 3B

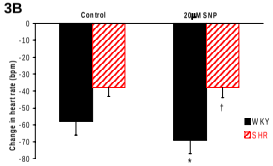


Figure 3A. Raw data trace showing the effect of 20μmol/L SNP on the heart rate response to vagal stimulation at 10Hz in a SHR and WKY rat atrial preparation.
Figure 3B. SNP significantly enhanced the response at 10Hz in WKY (n=7) but not SHR (n=6) preparations ($p<0.05$). SNP significantly increased baseline heart rate by a similar amount in both WKYs (+49±7, n=8) and SHRs (+43±6, n=6). SNP significantly enhanced the HR response to bath applied CCh (200 and 500nmol/L) to a similar extent in SHR and WKY rats.

Figure 4A

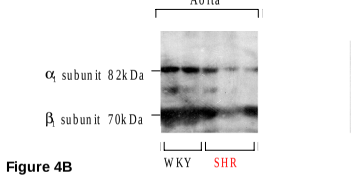


Figure 4B

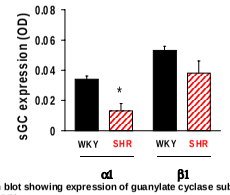


Figure 4A. Western blot showing expression of guanylate cyclase subunits in the aorta of SHR and WKY rats.
Figure 4B. The levels of α_1 and β_1 subunit expression were lower in the aorta of SHRs compared to WKYs.

Figure 5

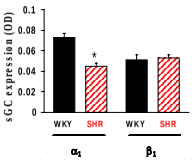
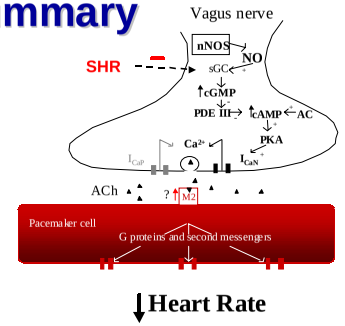


Figure 5. Expression of α1 subunits in the atria of SHRs (n=3) compared to WKYs (n=3). Expression of α1 subunits was 38% lower in SHRs but there was no change in β_1 expression.

Summary



Conclusions

There is an impaired chronotropic response to peripheral vagal nerve stimulation in hypertensive (SHR) compared with normotensive (WKY) rats.

This may result from altered pre-synaptic signalling, since the heart rate response to bath applied CCh was not attenuated in SHRs compared to WKYs.

This impairment is associated with down regulation of the α_1 subunit of guanylate cyclase in the SHR. However, the mechanism by which hypertension interferes with guanylate cyclase expression is not yet known.

The reduced vagal tone demonstrated in this study in the hypertensive rat may contribute to the overall increased risk of sudden cardiac death in hypertensive patients since low vagal tone is a negative prognostic indicator of clinical outcome[5].

References

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Acknowledgements

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