Expression of NO synthase and guanylate cyclase in the NTS during hypertension

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INTRODUCTION

- Hypertension is associated with impairment of the nitric oxide (NO)–cGMP pathway (Bauersachs 1998) and a reduced cardiac vagal drive in both human and animal models (Petretta, 1995; Murphy, 1991).

- Nitric oxide generated from endothelial nitric oxide synthase (NOS-3) in the nucleus tractus solitarius (NTS) inhibits the cardiac cholinergic baroreflex (Paton *et al.* 2001) whereas NO generated from neuronal nitric oxide synthase (NOS-1) in the cholinergic ganglia facilitates acetylcholine release and bradycardia (Choate *et al*, 2001 , Herring & Paterson, 2001). These opposing effects highlight the importance of NOS microdomains (see Barouch et al 2002) in the differential regulation of cardiac parasympathetic function.

- Functionally, the role of NO in the NTS of hypertensive rats is controversial. Pontieri *et al.* (1998) found no effect of inhibiting NOS activity in the NTS on baroreceptor reflex gain whereas an increase was observed by Kumagai *et al.* (1993). Therefore, it is possible that upregulation of eNOS in the NTS underlies the depressed baroreceptor reflex gain during hypertension.

AIMS

To examine patterns of NTS and cortex gene expression of nitric oxide synthases (eNOS and nNOS), guanylate cyclase (α1-GC, β1-GC) and superoxide dismutases (MnSOD, CuZnSOD) during hypertension.

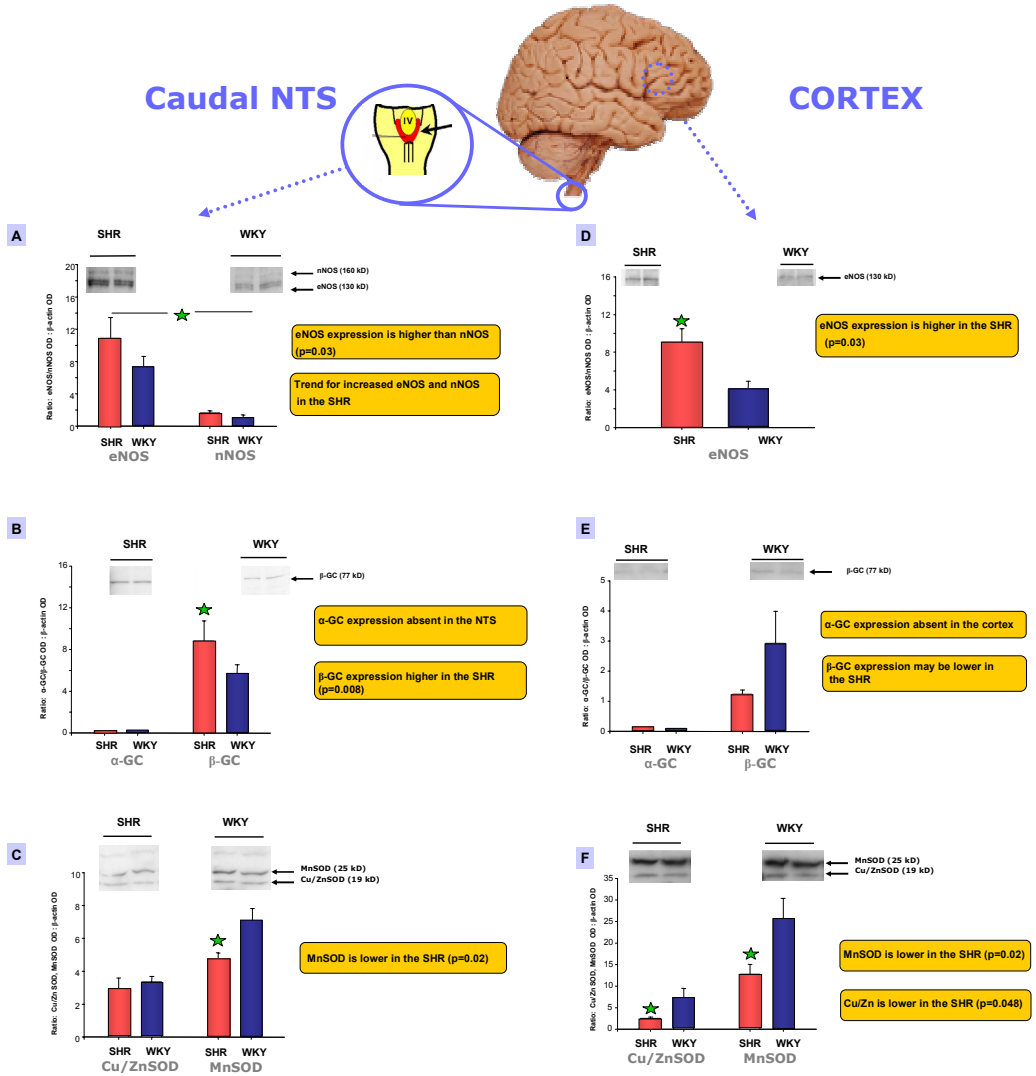
METHODS

Western Blotting

Patterns of gene expression were examined by Western blot analysis after micropunctates were cut out of the caudal NTS and cortex samples from 6-8 wk old spontaneously hypertensive rats (SHR, n=6) and normotensive Wistar-Kyoto rats (WKY, n=6).

1. Frozen samples were freeze-pulverised, homogenised and lysed in buffer containing a mammalian protease inhibitor mix (Sigma UK, P-8340).
2. Isolated protein (12.5μg in the NTS, 25μg in the cortex) was separated by gel electrophoresis, transferred to PVDF membranes and probed with specific antibodies using standard techniques.
3. Antibody-bound proteins were detected using luminol-based chemiluminescence and exposure to autoradiography film for 10 to 60 minutes. Autoradiographs were digitised and relative band densities determined. Between group comparisons were made using unpaired t-tests ($p<0.05$, ★) and expressed as a ratio to the amount of β-actin loaded in each lane.

RESULTS: Expression of NOS, Guanylate Cyclase and SOD



SUMMARY

		Fold change in the SHR relative to the WKY (x)
NTS		
	eNOS	1.5x
	nNOS	1.6x
	alpha-GC	no exp
	beta-GC	1.5x
	MnSOD	0.7x
	CuZn/SOD	0.9x
Cortex		
	eNOS	2.2x
	alpha-GC	no exp
	beta-GC	0.42 ** (low sample number)
	MnSOD	0.5x
	CuZn/SOD	0.6x

CONCLUSION

Our results suggest that the NO-cGMP pathway in the caudal NTS may be upregulated in the SHR, and thus provide a molecular substrate for inhibiting the baroreflex gain at the level of the caudal NTS in the SHR.

Furthermore, the role of the superoxide scavengers (MnSOD, Cu/Zn SOD) cannot be underestimated as they are downregulated during hypertension.

(Functional support for these findings are found in the poster by Waki et al.)

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