Hypertension in Spontaneously Hypertensive Rats occurs despite low plasma levels of homocysteine

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Key Words: amino acids, arginine, citrulline, homocysteine, hypertension, Ion Exchange Chromatography, nitric oxide, ornithine, Spontaneously Hypertensive Rats (SHR)

Short title: Low homocysteine levels in Spontaneously Hypertensive Rats

Word count: 1130 (summary 209)

Summary

Hyperhomocysteinemia has been suggested to induce hypertension due to its role in endothelial dysfunction. However, it remains controversial whether homocysteine and hypertension are truly causally related or merely loosely associated. To test the hypothesis that hyperhomocysteinemia occurs in Spontaneously Hypertensive Rats (SHR) we measured plasma levels of homocysteine in 10 male adult SHR and in 10 normotensive controls using Ion Exchange Chromatography. In addition, plasma concentrations of the 22 most common amino acids were measured to explore the relation of homocysteine with amino acid metabolism. Plasma levels of homocysteine were significantly lower in SHR (4.1µmol/L ± 0.1) than in controls (7.2 µmol/L ± 0.3;$p<0.00001$). The amounts of aminobutyric acid (ABU), alanine, citrulline and valine were decreased as well, whereas we found increased levels of aspartate, glutamate, glutamine, histidine and ornithine. Thus, contrary to our hypothesis, hypertension in SHR occurs despite low plasma levels of homocysteine. We provide a new hypothesis whereby reduced conversion of arginine to citrulline is related to increased ornithine levels, but decreased bioavailability of nitric oxide, resulting in impaired blood vessel relaxation and hypertension. In conclusion, our findings do not necessarily exclude that homocysteine and hypertension might be pathophysiologically connected, but corroborate the notion that hypertension can arise due to mechanisms independent of high homocysteine levels.

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Elevated homocysteine levels are an independent risk factor for atherosclerosis, ischemic stroke (Hankey and Eikelboom 2001), dementia and Alzheimer disease (Seshadri *et al.* 2002). Hyperhomocysteinemia has been suggested to evoke hypertension due to its role in endothelial dysfunction (Rodrigo *et al.* 2003). Possible mechanisms involve increased oxidative injury to the endothelium, proliferation of vascular smooth muscle cells and inhibition and degrading of arterial structural components such as collagen, elastin and proteogylcans (Rodrigo *et al.* 2003). In addition, high levels of homocysteine possibly decrease bioavailability of nitric oxide – also termed endothelium-derived relaxing factor - and thereby impair vasodilatation (Zhang *et al.* 2000). Indeed, higher homocysteine concentrations are associated with more advanced systemic arterial stiffness and greater blood pressure response to stress in hypertensive patients (Tayama *et al.* 2006) and healthy volunteers (Brett *et al.* 2006). However, it remains controversial whether homocysteine and hypertension are truly causally related or hyperhomocysteinemia merely is an indicator of vascular pathology (Dinavahi *et al.* 2004). To test the hypothesis that hyperhomocysteinemia occurs in the hypertension model of Spontaneously Hypertensive Rats (SHR) we measured plasma levels of homocysteine in 10 male adult SHR and in 10 normotensive controls (Wistar-Kyoto Rats) using Ion Exchange Chromatography. In addition, plasma concentrations of the 22 most common amino acids were measured to explore whether amino acid derangements other than hyperhomocysteinemia occur in SHR and might be linked to a hypothetical mechanism of hypertension largely independent of homocysteine.

All animal procedures were approved by the Swedish Animal Research Authority. Ten 26 weeks old male SHR and 10 normotensive aged-matched controls (Wistar-Kyoto Rats) were purchased from Charles River Laboratories, Germany. Prior to experiments the animals were

allowed to accommodate 14 days to their new surroundings, had free access to food and water and were kept five per cage at a light/dark cycle of 12 h, humidity 60%, temperature 22ºC. On the day of the experiment, the animals were sacrificed by decapitation. Blood was collected from the cranial vessels, immediately centrifuged and the plasma stored at -80°C until analysis. Plasma levels of aminobutyric acid (ABU), alanine, arginine, aspartate, citrulline, glutamate, glutamine, glycine, histidine, homocysteine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, taurine, threonine, tryptophane, tyrosine and valine were determined in sulfosalicylic acid deproteinized samples by Ion Exchange Chromatography using an JLC-500/V AminoTac™ Amino Acid Analyzer with photometric detection after reaction with ninhydrin (JEOL Ltd., Tokyo, Japan). A series of amino acid solutions from Beckman Coulter, Inc., was used as reference amino acid standards (Beckman Coulter, Inc., Beckman, CA). The coefficient of variation for all measurements was <10%. Statistical differences between groups were analyzed by the student's t-test and $p<0.05$ was considered significant.

Results are depicted in Table 1. Plasma levels of homocysteine were significantly lower in SHR (4.1µmol/L ± 0.1) than in controls (7.2µmol/L ± 0.3;$p<0.00001$). The amounts of ABU, alanine, citrulline and valine were decreased as well, whereas we found increased levels of aspartate, glutamate, glutamine, histidine and ornithine. Concentrations of arginine, glycine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, taurine, threonine, tryptophane and tyrosine remained unchanged (data not shown).

In the present study we found significant alterations in several of the naturally occurring amino acids. The decrease in citrulline and the increase in ornithine levels in SHR deserve special attention, since these amino acids are closely linked to the activity of nitric oxide synthase and hence, nitric oxide (NO). NO is produced from the reactions catalyzed by the

calcium-dependant NO synthase in which the precursor amino acid arginine is converted into citrulline. Impaired NO activity leads to reduced vasodilatation and hypertension (Rodrigo *et al.* 2003, Zhang *et al.* 2000) and has been documented in various rodent models of hypertension (Khunes *et al.* 2004). Contrary to our primary hypothesis, hypertension in SHR occurs despite low plasma levels of homocysteine. Homocysteine is a sulphur-containing amino acid formed during the metabolism of the essential amino acid methionine (Rodrigo *et al.* 2003). Homocysteine may be converted again to methionine, or converted to taurine or cysteine via the transsulfuration pathway. The methionine cycle occurs in all tissues and provides for the remethylation of homocysteine, which conserves methionine and recycles methyltetrahydrofolate (Rodrigo *et al.* 2003). Catabolism of methionine includes the conversion of serine and homocysteine to cystathionine and water (Jhee and Kruger 2005). It remains unknown, why homocysteine in the present study was decreased to such a significant degree compared to normotensive controls. However, the unchanged levels of methionine, serine and taurine in our study support the concept that homocysteine metabolism possibly has only minor, if any relevance for the development of hypertension in SHR.

We therefore generated a new hypothesis whereby reduced conversion of arginine to citrulline may be related to increased amounts of ornithine and a disturbance in the nitric oxide synthase (NOS) pathway. Figure 1 shows schematically the deranged pathways of amino acid metabolism in SHR, which are hypothesized to evoke hypertension without involving homocysteine metabolism. Whether decreased NOS activity may come secondary to reduced conversion of arginine to citrulline, or vice versa, remains to be determined. Alternatively, there might be a primary impairment of ornithine transcarbamoylase, which catalyses the transfer of the carbamoyl group from carbamoylphosphate to ornithine resulting in citrulline (Fig. 1). Although seemingly less arginine was converted to citrulline, arginine concentration remained unchanged. This was possibly due to enhanced conversion of arginine to ornithine, the level of which was significantly increased.

In conclusion, although still untested, our new hypothesis may provide an explanation for how homocysteine metabolism could be “by-passed” during the pathophysiologic processes that lead to hypertension in SHR. A short-coming of the present study is that NO levels were not measured. However, as mentioned above, impaired NO activity in rodent models of hypertension is well-documented (Khunes *et al.* 2004). Thus, our findings do not necessarily exclude that homocysteine and hypertension might be pathophysiologically connected, but support the notion that hypertension can arise due to mechanisms independent of high homocysteine levels. Moreover, the present results are in line with amounting evidence that treatment of hyperhomocysteinemia has no impact on cardio- and cerebrovascular morbidity (Bonaa *et al.* 2006, HOPE 2 2006, McMahon *et al.* 2006).

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Table 1

Concentrations (µmol/L) of amino acids in the plasma of Spontaneously Hypertensive Rats (SHR) and normotensive controls were measured using Ion Exchange Chromatography. Only significantly different values are shown.

	ABU	Alanine	Aspartate	Citrulline	Glutamate	Glutamine	Histidine	Homo-cysteine	Ornithine	Valine
Controls	10.8 ± 0.3	599 ± 15	23 ± 1.7	112 ± 2.5	134 ± 7.2	911 ± 23	87 ± 2.2	7.7 ± 0.2	66 ± 4.1	177 ± 3.6
SHR	9.1 ± 0.4**	439 ± 19**	36 ±3.5**	83 ± 1.4***	176 ± 7.1 **	1035 ± 25**	93 ± 1.8*	4.0 ± 0.0***	87 ± 8.9*	162 ± 4.5*

For details see Materials and Methods. The results are given in mean ± s.e.m. and were analyzed using the student's *t*-test. * $p<0.05$ ** $p<0.01$ *** $p<0.000001$

Figure 1

The figure shows schematically the deranged pathways of amino acid metabolism in SHR, which are hypothesized to evoke hypertension without involving homocysteine metabolism. For further information see *Discussion*.

↓ significantly decreased amounts

↑ significantly increased amounts

? possibly impaired enzymatic activity