SHORT COMMUNICATION

Hypertension in Spontaneously Hypertensive Rats Occurs Despite Low Plasma Levels of Homocysteine

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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 (4.1±0.1 µmol/l) than in controls (7.2±0.3 µmol/l) (p<0.00001). The amounts of aminobutyric acid, alanine, citrulline and valine were also decreased, 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.

Key words
Amino acids • Arginine • Citrulline • Homocysteine • Hypertension • Ion Exchange Chromatography • Nitric oxide • Ornithine • Spontaneously hypertensive rats (SHR)

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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 proteoglycans (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 is merely 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-week-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 22 °C. 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, alanine, citrulline and valine were also decreased, 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 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, to nitric oxide (NO) production. NO is produced from the reactions catalyzed by the calcium-dependent 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) as has been documented in various rodent models of hypertension (Kuneš 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

| 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. |

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Controls (WKY)</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminobutyric acid</td>
<td>10.8 ± 0.3</td>
<td>9.1 ± 0.4**</td>
</tr>
<tr>
<td>Alanine</td>
<td>599 ± 15</td>
<td>439 ± 19**</td>
</tr>
<tr>
<td>Aspartate</td>
<td>23 ± 1.7</td>
<td>36 ± 3.5***</td>
</tr>
<tr>
<td>Citrulline</td>
<td>112 ± 2.5</td>
<td>83 ± 1.4***</td>
</tr>
<tr>
<td>Glutamate</td>
<td>134 ± 7.2</td>
<td>176 ± 7.1**</td>
</tr>
<tr>
<td>Glutamine</td>
<td>911 ± 23</td>
<td>1035 ± 25**</td>
</tr>
<tr>
<td>Histidine</td>
<td>87 ± 2.2</td>
<td>93 ± 1.8*</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>7.7 ± 0.2</td>
<td>4.0 ± 0.0***</td>
</tr>
<tr>
<td>Ornithine</td>
<td>66 ± 4.1</td>
<td>87 ± 8.9*</td>
</tr>
<tr>
<td>Valine</td>
<td>177 ± 3.6</td>
<td>162 ± 4.5*</td>
</tr>
</tbody>
</table>

The results are given in mean ± S.E.M. and were analyzed by Student’s t-test. * p<0.05, ** p<0.01, *** p<0.000001.
pathway. The methionine cycle occurs in all tissues and provides 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. For further information see Discussion. ↓ significantly decreased amounts, ↑ significantly increased amounts, ? possibly impaired enzymatic activity.

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 shortcoming 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 (Kuneš 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 increasing evidence that treatment of hyperhomocysteinemia has no impact on cardio- and cerebrovascular morbidity (Bonaa et al. 2006, HOPE 2006, McMahon et al. 2006).

**Conflict of Interest**

There is no conflict of interest.

**References**


