H001

LOOP DIURETICS INTERFER WITH THE VASCULAR ACTIONS OF ENDOTHELIN-1 IN THE AORTA OF SPONTANEOUSLY HYPERTENSIVE RATS
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Torasemide is a loop diuretic with antihypertensive properties at non diuretical doses. In this study the ability of loop diuretics, torasemide and furosemide, to interfere with endothelin-1 (ET-1)-induced vascular responses in the aorta of spontaneously hypertensive rats (SHR) was investigated. The vasoconstrictor activity of ET-1 was examined in endothelium-denuded aortic rings from SHR using an organ bath system. ET-1-induced increases of intracellular calcium concentration ([Ca2+]i) were also examined by image analysis of cultured vascular smooth muscle cells (VSMCs) from SHR using fura 2 methodology. A dose-response curve to ET-1 was plotted for cumulative concentrations (from 10^-11 to 10^-6 mol/L) in endothelium-denuded aortic rings (pD2 = 9.1 ± 0.1). Isometric contraction induced by a submaximal concentration of ET-1 (10^-8 mol/L) was reduced in a dose-dependent way by torasemide and furosemide (IC50 values: 4 ± 1×10^-9 and 10 ± 2×10^-9 mol/L, respectively). Incubation of VSMCs with different concentrations of ET-1 (from 10^-8 to 10^-4 mol/L) resulted in a biphasic pattern, initial and sustained, of rise in [Ca2+]i (pD2: 7.0 ± 0.2 and 7.6 ± 0.6, respectively). The stimulatory effect of [Ca2+]i, induced by a submaximal concentration of ET-1 (10^-7 mol/L) was blocked by torasemide in both phases (IC50 = 0.4 ± 0.1 and 1.1 ± 0.1 μmol/L, respectively). Furosemide scarcely inhibited the initial rise in [Ca2+]i induced by ET-1, with no effect on the second rise. The present in vitro study shows that torasemide inhibits more potently the vasoconstrictor activity of ET-1 than does furosemide. This can be related to the fact that whereas torasemide blocks completely ET-1-induced increase of [Ca2+]i in VSMCs, furosemide inhibits it partially. Thus, the ability of loop diuretics to interfere with the vascular actions of ET-1 may involve different molecular mechanisms.

Key Words: Endothelin-1; furosemide; spontaneously hypertensive rats; torasemide

H002

INCREASED VASCULAR SUPEROXIDE PRODUCTION VIA NADH/NADPH OXIDASE ACTIVATION IN SPONTANEOUSLY HYPERTENSIVE RATS
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This study was designed to test the hypothesis that stimulation of nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate (NADH/NADPH) oxidase is involved in increased vascular superoxide anion (·O2−) production in spontaneously hypertensive rats (SHR). The study was performed in 16-week-old and 30-week-old normotensive Wistar-Kyoto rats (WKY16 and WKY30, respectively) and in 16-week-old and 30-week-old SHR (SHR16 and SHR30, respectively). In addition, 16-week-old SHR were treated with oral irbesartan (average dose 20 mg/Kg per day) for 14 weeks (SHR30-I). Aortic NADH/NADPH oxidase activity was determined by use of chemiluminescence with lucigenin. The expression of p22phox messenger RNA (mRNA) was assessed by competitive RT-PCR. Vascular responses to acetylcholine were determined by isometric tension studies. Aortic wall structure was studied determining the media thickness and the cross sectional area by morphometric analysis. Whereas systolic blood pressure (SBP) was significantly increased in the two groups of hypertensive animals compared with their normotensive controls, no differences were observed in SBP between SHR30 and SHR16. No other differences in the parameters measured were found between WKY16 and SHR16. In SHR30 compared with WKY30 we found significantly greater p22phox mRNA level, NADH/NADPH-driven ·O2− production, media thickness and cross sectional area, and an impaired vasodilation in response to acetylcholine. Treated SHR had similar NADH/NADPH oxidase activity and p22phox expression as the WKY30 group. The vascular functional and morphologic parameters were improved in SHR30-I. These findings suggest that an association exist between p22phox gene overexpression and NADH/NADPH overactivity in the aorta of adult SHR. Enhanced NADH/NADPH oxidase-dependent ·O2− production may contribute to endothelial dysfunction and vascular hypertrophy in this genetic model of hypertension.

Key Words: Endothelial dysfunction; NADH/NADPH oxidase; superoxide anion

H003

THE PRE-WEANING MATERNAL ENVIRONMENT DOES NOT MODULATE THE BP RESPONSE TO SALT IN THE SABRA RAT MODEL OF HYPERTENSION
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Background: Previous studies in SHR and Dahl rats have shown a significant impact of the maternal environment on the evolution of the BP phenotype. SHR raised by Wistar-Kyoto or Sprague-Dawley had attenuation of BP at 3 months. Similarly, Dahl S rats nurtured prior to weaning by Dahl R had a lower BP. In addition, in SHR and Dahl S, maternal environment has been demonstrated to have a significant impact on the BP response to salt.

Objectives: To test the hypothesis that the maternal environment immediately after birth modulates the expression of the salt-susceptibility genes and affects the BP response to salt-loading (salt-sensitivity and salt-resistance) in the Sabra rat model of hypertension.

Methods: The BP response to salt-loading was studied in SBH/y and SBN/y of both sexes in animals that had been...
nourished from immediately after birth and until weaning by a foster mother from the contrasting strain (reciprocal cross-fostering paradigm). Basal BP was measured shortly after weaning by the tail cuff method, animals were salt-loaded with DOCA-salt, and BP was measured again after 4 weeks.

Results: Data are provided as mean±sem, n=9–16 in each experimental (Exp) group, F-female, M-male. The basal BP

<table>
<thead>
<tr>
<th>Sex</th>
<th>Basal BP (mmHg)</th>
<th>SBN/y Exp – SBH/y</th>
<th>SBH/y Exp – SBN/y</th>
<th>Natural mother SBH/y</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Basal BP</td>
<td>121±2 125±2</td>
<td>138±1 126±1 128±1</td>
<td>139±1 140±1</td>
<td></td>
</tr>
<tr>
<td>after salt-loaded</td>
<td>128±1 128±1</td>
<td>196±2 193±2 132±6</td>
<td>195±2 192±3</td>
<td></td>
</tr>
</tbody>
</table>

and the BP response to salt-loading in female and male SBH/y and SBN/y raised by foster mothers of the contrasting strains were not different from those observed in animals of the same strains that had been raised by their natural mothers.

Conclusions: Expression of the salt-susceptibility genes is not affected by the maternal environment after birth in the Sabra model of salt-sensitive hypertension. The lack of such potentially confounding environmental modulator adds further strength to this model as highly suitable and focused for the search of the salt-susceptibility genes.

Key Words: Sabra rats; foster mother; experimental models; salt-sensitivity

H004

AGE DOES NOT AFFECT THE BLOOD PRESSURE RESPONSE TO SALT-LOADING IN THE SABRA RAT MODEL OF HYPERTENSION

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Background: Blood pressure (BP) is a quantitative trait that is affected by age in humans and in a variety of experimental animal models. Age is thus a potentially confounding factor in hypertension research, particularly in the determination and definitions of the BP phenotypes which may require age-adjustment.

Objectives: To test the hypothesis that the age of the animal modulates expression of the salt-susceptibility genes and affects the BP response to salt-loading (salt-sensitivity and salt-resistance) in the Sabra rat model of hypertension.

Methods: The blood pressure response to salt-loading was studied in SBH/y and SBN/y rats of both sexes at 1, 3, 6, 9 and 12 months of age. Basal BP [B] was measured by the tail cuff method, animals were salt-loaded with DOCA-salt, and BP was measured again after 4 weeks [S].

Results: Data are presented as mmHg, mean (n=5–6 per group). Basal BP was at all the ages studied 10–20 mmHg higher in SBH/y than in SBN/y. This difference was sustained up to age 1 year. Basal BP remained at similar values in both strains and sexes up to 1 year of age. Salt-loading in male and female SBN/y did not induce any significant increments in BP. Salt-loading in SBH/y induced, in contrast, a highly significant rise in BP of 40 mmHg or more at all the ages studied. There was no difference in the magnitude of the blood pressure response at all ages, and there were no differences between the sexes.

Conclusions: This study confirms that SBH/y do not develop spontaneous hypertension with age. The results further refute the hypothesis and demonstrate that salt-sensitivity in SBH/y and salt-resistance in SBN/y are not age dependent, are fully expressed from weaning on and at least until 1 year of age, do not require any specific age-window for their expression to be studied, and are not sex-dependent.

Key Words: Sabra rats; age; experimental models; salt-sensitivity

H005

COMPARISON OF SUPEROXIDE DISMUTASE GENE EXPRESSION AND ACTIVITY IN THE HEART OF SPONTANEOUSLY HYPERTENSIVE RATS WITH THAT IN NORMOTENSIVE RATS

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Objective: Membrane abnormalities in human essential hypertension are well-established. Recent study has suggested that oxygen free radicals (OFR) play a role in the pathogenesis of hypertensive vascular disease. Superoxide dismutase (SOD) is a naturally existed antioxidant in human which has an important role in scavenging OFR. In order to determine if changes in SOD in the heart occur in the hypertensive state.

Design and Methods: The present study compared the levels of the two main subtypes of this enzyme in spontaneously hypertensive rats (SHR) with age-matched normotensive Wistar-Kyoto (WKY) rats using enzyme activity estimation, Western blotting analysis for enzyme contents, and Northern blotting analysis of mRNA level at 6-week, 9-week and 12-week old rats.

Results: The systemic mean blood pressure in SHR was 184±12 mmHg which was significantly higher than 132±11 mmHg in WKY (n=8; p<0.001). Activity of Mn-SOD in SHR was significantly higher than WKY (12 weeks: SHR 55.9±0.8 vs WKY 33.6±0.4 unit/mg protein; p<0.01). The activity of CuZn-SOD of SHR hearts was also higher than WKY (12 weeks: SHR 31.9±0.4 vs WKY 27.3±0.4 unit/mg protein; P<0.05), similar results were found in mRNA levels.