Hypothalamic Substance P Release

Attenuated Angiotensin Responses in mRen2(27) Transgenic Rats

Debra I Diz, Burt Falgui, Susan M. Bosch, Brian M Westwood, Jessica Kent, Detlev Ganten, Carlos M Ferrario

Abstract

Increases in arterial pressure and paraventricular nucleus vasopressin release in response to intracerebroventricular injections of angiotensin peptides are blunted in mRen2(27) transgenic (TG+) rats. Intraventricular injections of tachykinin peptides mimic several of the actions of angiotensin peptides, and angiotensin peptides evoke substance P release from hypothalamic brain slices. The present study assessed whether diminished substance P release occurs in response to angiotensin peptides in TG(+) rats compared with normotensive control (TG(-)) rats. Systolic blood pressure at 8 to 12 weeks of age averaged 197±24 mm Hg (n=20, P<0.05) in TG(+) rats compared with 123±24 mm Hg in normotensive control (TG(-)) rats (n=18). Body weight was lower in hypertensive than in normotensive rats (305±14 versus 344±13 g, respectively, P<0.05).

Brain slices from hypothalamus were perfused at 37°C with oxygenated Krebs' bicarbonate buffer. Substance P was measured before (basal) and during perfusion with either Krebs' buffer (control) or 2 μmol/L angiotensin-(1-7) or angiotensin II. Basal substance P release was 92±10 pg/g wet tissue in TG(+) and 98±12 pg/g in TG(-) rats (P>0.05). Angiotensin-(1-7) and angiotensin II significantly increased substance P release from hypothalamus of TG(-) rats (82% and 70% above control, P<0.05) but not TG(+) rats. These studies further support the hypothesis that the cardiovascular effects of angiotensin peptides are mediated in part by substance P and that this relationship is blunted in a hypertensive model that results from excess tissue production of angiotensins.

Key Words: angiotensin peptides • substance P • hypothalamus • transgenic rat • hypertension

Recent studies indicate that the mRen2(27) transgenic rat [TG(+)], which expresses high levels of angiotensin peptides in the brain, is less responsive to exogenously applied angiotensin peptides than normotensive rats. For example, Morihuchi et al2 showed that the pressor response to intraventricular injections of angiotensin II was absent in TG(+) rats compared with normotensive Hannover Sprague-Dawley rats. Hypothalamic release from hypothalamic nuclei in response to either angiotensin-(1-7) or angiotensin II was also diminished in the TG(+) rats compared with the normotensive control rats. The attenuated responsiveness does not appear to be a result of a reduction in the apparent binding density of angiotensin II in brain or peripheral tissues. However, additional studies in TG(+) do suggest a downregulation of the receptor signaling pathways linked to angiotensin II receptors in both brain and periphery that is not accounted for by reduced numbers of receptors.

In earlier studies we showed that in brain slices from the hypothalamus, both angiotensin II and angiotensin-(1-7) stimulated the efflux of substance P. Substance P and other tachykinin peptides exhibit cardiovascular actions consistent with regulation of fluid and electrolyte balance at hypothalamic sites. These actions include increases in arterial pressure and release of vasopressin after intraventricular or paraventricular injections. In addition, interactions among the effects of angiotensin and tachykinin peptides are becoming increasingly apparent. We showed that the cardiovascular actions of angiotensin II injected into the nucleus of the solitary tract are blocked by an antagonist of substance P. Others report that the vasopressin release and hypothalamic pressor actions of tachykinins are mediated by angiotensins.

This study was designed to assess both basal and angiotensin peptide-stimulated release of substance P from hypothalamic slices prepared from TG(+) rats compared with that from normotensive control rats. Our findings suggest that the reduced cardiovascular effects and vasopressin release in TG(+) rats in response to exogenously administered angiotensin peptides are associated with augmentation of angiotensin peptide-stimulated release of substance P in the hypothalamus as well.

Methods

Male heterozygous mRen2(27) transgenic rats (8 to 12 weeks of age) and age-matched normotensive control rats both derived from Hannover Sprague-Dawley rats were obtained from the breeding colony of the Hypertension Center at Bowman Gray School of Medicine. Rats were genotyped using polymerase chain reaction amplification for the presence of the mRen2(27) gene according to previously published methods to distinguish littermates carrying the mouse renin gene [TG(+)] and those without the gene [TG(-)]. Tail-cuff systolic blood pressures were obtained on untrained rats in the week before study by using a Narco Bio-Systems apparatus (Division of International Bio-Medical, Inc). Rats were housed in an AAALAC-approved temperature- and humidity-controlled room on a 12:12-hour light-dark cycle with free access to water and regular rat chow. All procedures involving animals adhere to institutional guidelines.
and were approved by the Institutional Animal Care and Use Committee.

Rats were decapitated, the brain removed rapidly, and the
hypothalamus dissected away from surrounding tissue according
to the following landmarks on the ventral surface 1 mm rostral to
the optic chiasm, caudally at the junction of the median eminence,
and laterally within 1 mm of the interface with the cortex Dor-
sally, the cortical tissue above the ventricles was removed This
tissue block was then shread into 1-mm slices in two directions
and placed into a chamber filled with 1 mL of Krebs' solution
containing 20 μmol/L bacitracin and 6 μmol/L dithio-
threitol (Sigma) and bubbled with 95% O2-5% CO2 to a pH of
7.4, as reported previously 9. The tissue in the chamber was perfused with oxygenated Krebs' solution for 16 minutes to allow for equilibration. Subsequently, three 6-minute collections were taken (=2.5 mL volume each) The first collection period was used as an index of basal efflux of substance P. The second collection period (stimulus period) consisted of continued perfusion with either buffer only (control) or buffer containing 2 μmol/L of either angiotensin II or angiotensin-(1-7) (Bachem). During the third collection period, all tissues were perfused with buffer only for a recovery period At the end of the experiment, the tissue was removed from the chamber, blotted dry, and weighed.

For substance P measurements, 1 mL of each sample was added to tubes containing 50 μL of 19 mg/mL aprotonin (Sigma) and 68 μL of 5 μmol/L EDTA, lyophilized, and reconstituted to a volume of 0.2 mL for assay. The samples were measured by radioimmunoassay (ICSTAR Corporation). Values below the detection limit were assigned the detection limit of 4 pg/mL. Cross-reactivity of the antibody with other tachykinin peptides (physalaemun, eledoisin), as reported by the manufacturer of the assay kit was less than 0.002%. Interassay variability averaged 15%. In preliminary studies, the buffer either alone or containing the inhibitors, and buffer containing 2 μmol/L angiotensin II or angiotensin-(1-7) was tested for cross-reactivity or interference in the substance P assay. Values were below the detection limit for each of these determinations.

Basal release values were defined as the amount of spontaneous release in the first 6-minute period and expressed per gram tissue wet weight and per milliliter of perfusate. Responses over time (stimulus and recovery periods) are expressed as percent of basal If basal values and values from subsequent periods were below the detection limit, the data were excluded from analysis since the percent change from basal could not be determined.

Values are reported as mean±SEM. Unpaired Student's t tests were used to compare pressure, body weights, and basal release between TG(+) and TG(-) rats. Data were evaluated using two-way analyses of variance (ANOVA) for between-strain [TG(+) versus TG(-)] comparisons, followed by Mann Whitney tests. One-way ANOVA followed by Student-Newman-Keuls post hoc comparisons was used to compare among treatment groups in a given rat strain [Control, angiotensin II, angiotensin-(1-7)] in the stimulus and recovery periods. Logarithmic transformation of the data was performed to achieve homogeneity of variance. InStat and Prism software were used for these analyses (GraphPad). A value of P<0.05 was taken as indicative of a significant difference among means.

### Results

#### Comparison of Initial Values in TG(+) and TG(-) Rats

Systolic blood pressure and body weights for the TG(+) and TG(-) animals used in this study are shown in the Table. Systolic pressure was significantly higher and body weight lower in TG(+) rats than in the normotensive controls. Basal release of substance P was not different between hypertensive and normotensive animals when expressed on the basis of tissue weight or concentration in the perfusate (Table).

#### Differences in Responses to Angiotensin Peptides in TG(+) and TG(-) Rats

In both groups of rats, values for substance P in the perfusate tended to decline with time to ≈80% of basal as seen in the control group perfused with buffer only throughout the experiment (see the Figure) In the presence of either angiotensin II (2 μmol/L) or angiotensin-(1-7) (2 μmol/L) during the stimulus period, there was a 70% to 80% increase in substance P efflux above the time control group from the hypothalamic slices of TG(-) rats (F=4.328, P<0.036) On the other hand, there was no sig-

### Substance P Release

**Table**

<table>
<thead>
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<th>TG(+) (n=20-21)</th>
<th>TG(-) (n=17-18)</th>
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<tbody>
<tr>
<td>Systolic pressure, mm Hg</td>
<td>197±4*</td>
<td>122±4</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>305±14*</td>
<td>345±14</td>
</tr>
<tr>
<td>Basal substance P release</td>
<td>90±10</td>
<td>90±12</td>
</tr>
<tr>
<td>pg/g</td>
<td>11±1</td>
<td>13±2</td>
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</table>

TG(+) indicates mRen2(27) renin transgenic rats, TG(-), normotensive control rats. Systolic blood pressure by tail cuff and body weight were determined in the week preceding the experiment. Basal substance P release values indicate the amount of immunoreactive substance P detected in the 6-minute collection period preceding the stimulus period. Values are expressed per gram of wet tissue weight (pg/g) or per milliliter of perfusate collected (pg/mL).

*P< 0.05, **P< 0.01 compared with TG(-) rats.
significant increase in substance P release from hypothalamic slices of the TG(+) rats treated with either angiotensin II or angiotensin-(1-7) (F = 106, P = 368). Furthermore, the increase in substance P in response to angiotensin II in TG(−) rats was significantly greater than that observed in TG(+) rats (P < 0.05). There was no statistical difference in the magnitude of the substance P response to angiotensin-(1-7) between hypertensive and normotensive rats.

In both peptide-treated groups values for substance P declined towards the control group in the recovery period. There were no differences among control and peptide-stimulated groups or between TG(+ ) and TG(−) rats during this period.

**Discussion**

Angiotensin II and angiotensin-(1-7) both stimulate the efflux of substance P from hypothalamic brain slices of normotensive TG(−) rats. These findings are consistent with our previous studies showing that both angiotensin peptides at the dose employed here caused an increase in substance P from hypothalamus of Harlan Sprague-Dawley rats. In contrast, there was no significant increase in substance P during stimulation with the same dose of either of the angiotensin peptides in the hypothalamic tissue from the TG(+) rats. Previous work by Morishita et al. indicated that the pressor response and vasopressin release produced by intraventricular administration of angiotensin II and the vasopressin release caused by intraventricular angiotensin-(1-7) were attenuated in TG(+) rats. Thus, the present study reveals yet another mechanism of decreased responsiveness to exogenously administered angiotensin peptides in the TG(+) rat.

The mechanism for the attenuation of the angiotensin peptide-induced responses in the TG(+) rat is not known. Endogenous levels of angiotensin II and angiotensin-(1-7) are substantially higher in the hypothalamus of TG(+) rats than in normotensive control rats. In spite of the high levels of angiotensin peptides, separate studies suggest that there are no differences in receptor density or affinity in various brain and peripheral areas. Therefore, downregulation of the receptor itself appears unlikely. However, recent studies by Tallant et al. and Wess et al. indicate that the activation of inositol phosphate metabolism by angiotensin II is diminished in cultures of brain astrocytes or vascular smooth muscle cells from TG(+) rats. In our study, basal substance P was not different between hypertensive and normotensive rats, but there was no significant increase in substance P in the TG(+) rats when the angiotensin peptides were tested at a single dose. Because additional doses were not tested in our study, it cannot be determined whether the reduced response represents a partial or complete receptor desensitization. Further insights can be obtained, however, from the data of Tallant and colleagues since a wider dose range (10−9 to 10−5) was used in their studies. They reported that the IC50 for angiotensin II was roughly the same in cells from both TG(+) and TG(−) rats. However, the maximal response was reduced by over 60%. Thus, it is possible that basal release of substance P reflects the presence of high endogenous peptide levels in the face of “normal” receptor numbers. The uncoupling of receptor-signaling mechanisms may then account for both the absence of differences in basal release and the failure of the angiotensin peptides to stimulate the efflux of substance P in TG(+) rats. The somewhat greater inhibition of the angiotensin II response than of that obtained with angiotensin-(1-7) in the TG(+) rats might be accounted for by the fact that the hypothalamic angiotensin II levels are elevated almost 10-fold in the TG(+) rats, whereas the angiotensin-(1-7) levels are only 5-fold higher. Alternately, these differences may reflect differences in signal transduction pathways activated by angiotensin II and angiotensin-(1-7).

Finally, these studies provide further information on the possible pathways and transmitters involved in the cardiovascular and fluid balance actions of angiotensin peptides. Recent work by us in the dorsal medulla oblongata of the rat revealed that the acute cardiovascular actions of angiotensin II are mediated by substance P, whereas release of other transmitters may be responsible for the pressor effects of angiotensin II. Evidence in support of this concept comes from a report by Unger et al. who showed that the mechanisms mediating the pressor effects of the substance P and angiotensin II differ. In addition, our earlier studies indicated that while angiotensin II increased substance P, dopamine and norepinephrine efflux from hypothalamic slices, angiotensin-(1-7) increased only substance P efflux. Central nervous system release of dopamine, serotonin or norepinephrine is known to contribute to the pressor effects of angiotensin II. Differences in the transmitters released in response to the two peptides provides a potential mechanism for the lack of pressor effects of angiotensin-(1-7).

In summary, the TG(+) hypertensive rat provides a model of hyperresponsiveness to exogenously administered angiotensin peptides. While the mechanism for the reduced response is not yet known, it is likely to be the result of the elevated endogenous peptides and a concomitant desensitization of the receptor signaling pathways.

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**References**

5 Tallant EA, Ganten D, Ferraro CM. Attenuated responses to angiotensin II in vascular smooth muscle cells from transgenic (mRen2)27 rats. Am J Hypertens 1994,7 1359 Abstract
6 Weiss RB, Ganten D, Ferraro CM, Tallant EA. Reduced response to Ang II in astrocytes isolated from neonatal brain of (mRen2)27 transgenic rats. Hypertension 1995,23 1410 Abstract
7 Diz DI, Bosch SM, Monguchi A, Ganten D, Ferraro CM. Converting enzyme inhibitor lowers angiotensin II receptor density in medulla of transgenic (mRen2)27 rats. J Hypertens 1994,12 1371 Abstract
9 Diz DI, Prro N. Differential actions of angiotensin II and angiotensin-(1-7) on transmitter release. Hypertension 1992,19(suppl II) I-II-48
10 Perfumi M, Massi M, Epstein AN, DeCaro G. Neurokinin A is a specific and precocious inhibitor of water intake in neonatal rats. Peptides 1990,11 339-344
13 Diz DI, Funtz DL, Benter IF, Bosch SM. Substance P antagonist blocks the cardiovascular effects of angiotensin II in the rat nucleus tractus solitarii. Hypertension 1985,5 1360 Abstract
17 Freeman EJ, Ferraro CM, Tallant EA. Angiotensins differentially activate phospholipase D in vascular smooth muscle cells from spontaneously hypertensive and Wistar-Kyoto rats. Am J Hypertens 1995,8 1105-1111
19 Schuambe MT, Santos RAS, Brosnnhan KB, Khosla MC, Ferraro CM. Release of vasopressin from the rat hypothalamo-neurohypophyseal system by angiotensin-(1-7) heptapeptide. Proc Natl Acad Sci USA 1988,85 4095-4098
25 Kawabe H, Brosnnhan KB, Diz DI, Ferraro CM. Role of brain dopamine in centrally evoked angiotensin II responses in conscious rats. Hypertension 1986,8(suppl I) I-84-I-89
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