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) renin 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±4 mm Hg in TGI(+) rats compared with 123±4 mm Hg in normotensive control (TG(-)) rats. Body weight was lower in hypertensive than in normotensive rats (305±14 versus 344±13 g, respectively, P<0.05). Brain slices from hypothalamic slices 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 hypothalamic slices 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 angiotensin (Hypertension. 1997;29[part 2]:510-513.)

Key Words • angiotensin peptides • substance P • hypothalamus • renin 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, Moruguchi et al showed that the pressor response to intraventricular injections of angiotensin II in TG(+) rats compared with normotensive Hannover Sprague-Dawley rats is diminished in the hypothalamic nucleus in response to either angiotensin-(1-7) or angiotensin II. This 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.

This study was designed to assess both basal and angiotensin peptidem-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 attenuation 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 were obtained 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 using a Narco Bio-Systems apparatus (Division of International Biomedical, Inc.) Rats were housed in an AALAC-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 hypothalamic tissue 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. Dorsally, the cortical tissue above the ventricles was removed. This tissue block was then sliced into 1-mm slices in two directions and placed into a chamber filled with 1 mL of Krebs’ solution containing 20 μmol/L bacitracin (Sigma) and 6 μmol/L dithiothreitol (Sigma) and bubbled with 95% O2-5% CO2 to a pH of 7.4, as reported previously.

The tissue in the chamber was perfused with oxygenated Krebs’ solution for 15 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 (Ang II), 2 μmol/L angiotensin-(1-7) (Ang-(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 aprotinin (Sigma) and 6.8 μL of 0.5 mol/L EDTA, lyophilized, and reconstituted to a volume of 0.2 mL for assay. The samples were measured by radioimmunoassay (NCSTAR Corporation). Values below the detection limit were assigned the detection limit of 4 pg per tube. Cross-reactivity of the antibody with other tachykinin peptides (physalaemin, 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 of either angiotensin II or angiotensin-(1-7) (Bachem) 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. Comparisons were performed using the Student-Newman-Keuls post hoc test. 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 was used to compare among treatment groups in a given rat strain.
substantially higher in the hypothalamus of TG(+) rats. There were no differences in receptor density or affinity in the levels of angiotensin peptides, separate studies suggest that endogenous levels of angiotensin II and angiotensin-(1-7) are reported to be mediated by different mechanisms and/or receptors. It is tempting to speculate that the increase in substance P might result in the hypothalamic vasopressin-release effects of the two peptides, 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, dopamime and norepinephrine efflux from hypothalamic slices, angiotensin-(1-7) increased only substance P efflux. Central nervous system release of dopamine, serotonin or noradrenaline 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 hyporesponsiveness 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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