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Reduction of Blood Pressure by AT_1 Receptor Decoy Peptides

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ABSTRACT

Background: We previously identified the binding of the chaperone protein gamma-aminobutyric acid receptor–associated protein (GABARAP) to a sequence on the carboxy-terminus of the angiotensin II AT_1 receptor (AT_1R) and showed that this binding enhances AT_1R trafficking to the cell surface as well as angiotensin signaling.

Methods: In this study, we treated sodium-depleted mice with decoy peptides consisting either of a fusion of the cell-penetrating peptide penetratin and the GABARAP/AT_1R binding sequence or penetratin fused to a mutated AT_1R sequence. We used telemetry to measure blood pressure.

Results: Systolic and diastolic pressure fell during the 24 hours following decoy peptide injection but not after control peptide injection. Active cell-penetrating decoy peptide decreased 24-hour average systolic blood pressure from 129.8 $\pm$ 4.7 mmHg to 125.0 $\pm$ 6.0 mmHg (mean $\pm$ standard deviation). Diastolic blood pressure fell from 99.0 $\pm$ 7.1 mmHg to 95.0 $\pm$ 9.2 mmHg (n=5). Administration of the control peptide raised systolic blood pressure from 128.7 $\pm$ 1.3 mmHg to 131.7 $\pm$ 2.9 mmHg and diastolic pressure from 93.9 $\pm$ 4.5 mmHg to 95.9 $\pm$ 4.2 mmHg (n=5). The decreases in both systolic and diastolic blood pressure after active peptide administration were statistically significant compared to control peptide administration ($P<0.05$, two-tailed Wilcoxon rank-sum test).

Conclusion: These results indicate the physiological and potentially therapeutic relevance of inhibitors of GABARAP/AT_1R binding.

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INTRODUCTION

Angiotensin II (AngII), acting predominantly via its AT_1 receptor (AT_1R), plays important roles in the regulation of blood pressure (BP) and intravascular volume. AT_1's action is often targeted in the treatment of hypertension and other disorders.[1-12] We previously identified the binding of the chaperone protein gamma-aminobutyric acid receptor–associated protein (GABARAP) to a sequence on the carboxy-terminus of the AT_1 receptor (AT_1R) and showed that this binding enhances AT_1R trafficking to the cell surface as well as angiotensin signaling.[13,14] To determine the effect of inhibiting receptor/chaperone interaction in vivo, we treated sodium-depleted mice with decoy peptides consisting of either a fusion of the cell-penetrating peptide (CPP) penetratin and the GABARAP/AT_1R binding sequence of AT_1R or a fusion of penetratin and a mutated AT_1R sequence. We used telemetry to measure BP.

METHODS

C57B16/J male mice approximately 6 months of age (Jackson Laboratories, Bar Harbor, ME) were bred in house and placed on a low-sodium diet—Teklad, 0.01-0.02% NaCl (Harlan Laboratories, Indianapolis, IN)—for 19 days in order to induce AngII BP dependence.

Imgenex (San Diego, CA) custom engineered the fusion peptides. The active decoy peptide—CPP-1—was a fusion of penetratin with GKKFKKYFLQL (AT_1R). The control decoy peptide (CPP-2) was a fusion of penetratin with a mutated GABARAP/AT_1R binding site sequence (or GKKFEEAFLQL). We injected the peptides using a chronically implanted jugular cannula at 0 and 8 hours (23 μg of peptide in a total

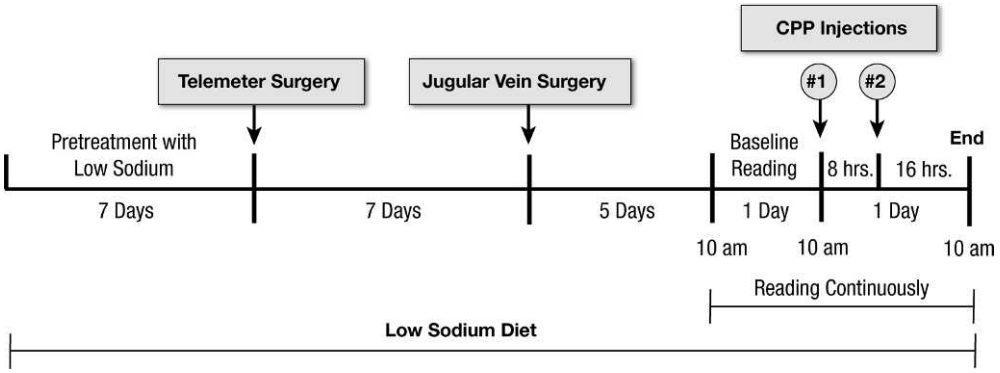

Figure. Cell-penetrating peptide (CPP) injection timeline for mice. Mice were placed on a low sodium diet (Teklad, 0.01-0.02% NaCl, Harlan Laboratories, Indianapolis, IN), implanted with blood pressure telemeters, and injected via the jugular vein with active (CPP-1) or mutated inactive (CPP-2) peptide as described in the text. Blood pressure was measured via mouse telemeter in the aorta after left carotid artery catheterization. Pressure was measured every 30 minutes for 24 hours after CPP injection using a Physiotel PA series transmitter (model PA-C10) and the Dataquest ART 4.1 Data Acquisition and Analysis System (Data Sciences International, St. Paul, MN).

volume of 250 μL). We monitored BP continuously by telemetry from 24 hours prior to injection until 24 hours after the initial administration of the decoy (CPP-1) or control (CPP-2) peptides. The figure outlines the experimental design.

RESULTS

CPP-1 decreased 24-hour average systolic BP from 129.8 ± 4.7 mmHg to 125.0 ± 6.0 mmHg (mean ± standard deviation). Diastolic BP fell from 99.0 ± 7.1 mmHg to 95.0 ± 9.2 mmHg (n=5). CPP-2 raised systolic BP from 128.7 ± 1.3 mmHg to 131.7 ± 2.9 mmHg and diastolic BP from 93.9 ± 4.5 mmHg to 95.9 ± 4.2 mmHg (n=5). The decreases in both systolic and diastolic BP after administration of the active peptide were statistically significant compared to changes after administration of the control peptide ($P<0.05$, two-tailed Wilcoxon rank-sum test).

DISCUSSION

AngII is the major effector protein of the renin-angiotensin system. It acts on vascular smooth muscle cells to induce vasoconstriction and on adrenal cortical cells to stimulate aldosterone secretion. Both of these actions increase BP. The peptide also can bind to receptors in the brain and affect the neural control of BP. Therefore, AngII is an important factor in the maintenance of normal BP as well as in the pathogenesis of hypertension.[1-6] Indeed, drugs designed to reduce the production of AngII (direct renin inhibitors and converting enzyme inhibitors) or to block its action at AT_1R—AngII's predominant cellular receptor—(angiotensin-receptor blockers) are widely used antihypertensive agents. Also, AngII is directly involved in the production of atherosclerosis, cardiac hypertrophy, congestive heart failure, and diabetic nephropathy and other renal diseases. As noted, the majority of AngII-mediated physiological actions occur through binding to the AT_1R, a 7-membrane–spanning G protein–coupled receptor.[1-6]

AT_1Rs are not static on cell surfaces but can be internalized after AngII binding and either recycled to the cell surface or trafficked to other intracellular compartments. Chaperone proteins can modulate the trafficking of AT_1R to and from the cell surface. One chaperone protein is the angiotensin receptor–associated protein (ATRAP) that reduces trafficking of the receptor to the cell surface. The upregulation of ATRAP by physiological means can lessen the ability of hypertension to damage the kidneys of Dahl salt-sensitive rats, presumably by reducing AT_1R sites on renal cells and thereby limiting the harmful action of AngII on these cells.[7,8] A second chaperone protein is ARAP1 that, unlike ATRAP, facilitates trafficking of AT_1R to the cell surface.[9-12]

We discovered that another chaperone protein, GABARAP, like ARAP1, binds to the carboxy-terminus of AT_1R and promotes the trafficking of the receptor to the cell surface.[13,14] GABARAP has been known to affect gamma-amino butyric acid receptor trafficking and clustering in brain neurons but previously was not known to interact with AT_1R. In cell cultures, cotransfection of PC-12 cells (a pheochromocytoma cell line) with a fluorescent AT_1R fusion protein and GABARAP increased AT_1R cell surface expression by 6-fold. GABARAP overexpression in CHO-K1 cells that also

expressed AT_1R increased cell surface AngII binding more than 3-fold and increased AngII-driven signaling and proliferation as well. Knockdown of GABARAP with small interfering RNAs reduced AT_1R surface protein and binding.[13]

We next identified GABARAP/AT_1R interacting sites on each protein and a target sequence in the carboxy-terminus of AT_1R (GKKFKKYFLQL). We transfected mammalian cells with AT_1R and GABARAP and then, in a proof-of-concept experiment, treated the cells externally with cell-penetrating decoy peptides.[13,15] These peptides consisted of fusions of penetratin with GKKFKKYFLQL (AT_1R) or GKKFEEAFLQL (mutated AT_1R). The active peptide is designated CPP-1, and the control peptide is CPP-2. Deconvolution microscopy and immunoblot studies showed that the active decoy CPPs blocked GABARAP-induced AT_1R accumulation at the cell surface and blocked AngII-induced stimulation of phospho–extracellular signal-regulated protein kinases 1 and 2 by about 5-fold. CPPs fused to mutant AT_1R sequences had no effect.[15]

Research has shown AngII to be necessary to maintain normal BP in sodium-depleted, but not sodium-replete, humans.[16] We designed the present study to demonstrate the in vivo BP-lowering action of inhibitors of the GABARAP/AT_1R interaction by treating sodium-depleted mice with GABARAP/AT_1R decoy peptides.

This study demonstrates the in vivo relevance of the GABARAP/AT_1R interaction for BP and demonstrates the efficacy of decoy CPPs in lowering BP. This finding suggests that small molecule inhibitors could be developed to block the GABARAP/AT_1R interaction site and lower BP, as well as potentially reduce other harmful effects of AngII by actions at arterial smooth muscle or other sites.[1,4-6] Although the pressure reduction achieved in this study was modest, it was comparable to average pressure decreases obtained through the use of angiotensin-converting enzyme inhibitors in several large clinical trials.[17] Moreover, the decrease in BP reported here occurred in normotensive animals. Greater decreases would be expected in hypertensive animals, particularly animals with high renin hypertension. Like angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, these peptides or their small molecule analogues could find wide application in treating congestive heart failure, diabetic renal disease, and other disorders.

Because large proteins such as the decoy peptides described here do not cross the blood-brain barrier, the effects we observed are likely related to AT_1R in the cardiovascular system, suggesting that a different spectrum of activity could result from the delivery of these decoy peptides or their small molecule analogues into the central nervous system.

Additionally, the active decoy peptide described here lowers cell surface receptor number and, thereby, not only reduces AngII signaling but also reduces AT_1R-mediated AngII internalization and therefore any effects attendant upon that internalization.[1-4,18] Similarly, to the extent that constitutive AT_1R activity requires trafficking to the cell membrane, interruption of the GABARAP/AT_1R interaction by decoy peptides would be expected to blunt that activity.[19]

CONCLUSION

To our knowledge, this is the first report of a study showing that the inhibition of a chaperone protein binding to AT_1R can lower BP in vivo. This observation potentially has considerable therapeutic implications.

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