Published in final edited form as:

Physiol Behav. 2016 August 1; 162: 141–146. doi:10.1016/j.physbeh.2016.01.020.

Angiotensin II (de)sensitization: fluid intake studies with implications for cardiovascular control

Derek Daniels

Department of Psychology, Behavioral Neuroscience Program, University at Buffalo, the State University of New York, Buffalo NY 14260 USA

Abstract

Cardiovascular disease is the leading cause of death worldwide and hypertension is the most common risk factor for death. Although many anti-hypertensive pharmacotherapies are approved for use in the United States, rates of hypertension have increased over the past decade. This review article summarizes a presentation given at the 2015 meeting of the Society for the Study of Ingestive Behavior. The presentation described work performed in our laboratory that uses angiotensin II-induced drinking as a model system to study behavioral and cardiovascular effects of the renin-angiotensin system, a key component of blood pressure regulation, and a common target of anti-hypertensives. Angiotensin II (AngII) is a potent dipsogen, but the drinking response shows a rapid desensitization after repeated injections of AngII. This desensitization appears to be dependent upon the timing of the injections, requires activation of the AngII type 1 (AT₁) receptor, requires activation of mitogen-activated protein (MAP) kinase family members, and involves the anteroventral third ventricle (AV3V) region as a critical site of action. Moreover, the response does not appear to be the result of a more general suppression of behavior, a sensitized pressor response to AngII, or an aversive state generated by the treatment. More recent studies suggest that the treatment regimen used to produce desensitization in our laboratory also prevents the sensitization that occurs after daily bolus injections of AngII. Our hope is that these findings can be used to support future basic research on the topic that could lead to new developments in treatments for hypertension.

Keywords

Angiotensin; hypertension; water intake; saline intake; sensitization; desensitization

Correspondence and Reprint Request to: Derek Daniels, Department of Psychology, University at Buffalo SUNY, Buffalo, NY 14260, United States. Tel.: 716-645-0264; Fax: 716-645-3801; danielsd@buffalo.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosures: none

Introduction

The annual meeting of the Society for the Study of Ingestive Behavior (SSIB) is heavily focused on feeding behavior. Although the name of the SSIB indicates that behavior is a primary focus, the inclusion of studies of metabolic disorders, including obesity, are welcomed at the meeting because of the general acceptance that these disorders are connected to feeding behavior, at least in some respects. Studies on fluid intake have become underrepresented at the annual meeting over the past several decades, not because these studies have been purposefully excluded from the scientific program, but because the number of scientists studying fluid intake seems to have decreased, whereas the number of scientists studying food intake seems to have increased. Nevertheless, each year, the annual SSIB meeting features some studies of fluid intake.

The relationship between obesity and food intake is analogous to the relationship between hypertension and fluid intake. Obesity results from a perturbation of energy homeostasis, whereas hypertension is a consequence of a perturbation of body fluid homeostasis. In this sense, studies on cardiovascular control and cardiovascular disease should be welcomed by the SSIB in the same way that studies of obesity and other metabolic disease states are featured at the meeting. From the SSIB's perspective, these topics are relevant because of their relationship to ingestive behavior, but from a more general perspective, these topics are interesting because our research has the potential to improve health. This improvement matters to us, as human beings, because death is inevitable and it is natural to try to postpone that inevitability for as long as possible.

When we consider the causes of death, we can approach it from at least two perspectives: from a likely cause perspective (what is the most common cause of death) or from a risk factor perspective (what are the factors that best predict death). With respect to the former, cardiovascular disease is the leading cause of death in the United States [1] and worldwide [2]. An analysis of deaths in 187 countries in 2010, for instance, reports that of the 52.8 million deaths included in the analysis, 15.6 million of them (29.5%) were due to some form of cardiovascular disease [2]. From a risk factor perspective, hypertension is the single greatest risk factor for death [3, 4]. In 2010, for example, 9.4 million deaths worldwide were attributable to high blood pressure [4] (Figure 1, top).

Rates of hypertension among adults in the United States have risen significantly over the past decade (Figure 1, bottom). This rise has occurred in spite of several FDA-approved treatments. These treatments are clearly imperfect, and do not work for every patient. Indeed, data from NHANES 2007–2010 indicate that a sizable proportion of the drug-treated US adult hypertensive population is classified as treatment resistant [5]. This is not surprising because of the complex systems that regulate pressure, and the compensatory mechanisms that can be engaged when pressure is high or low. Indeed, regulation of blood pressure requires constant coordination of numerous bodily systems ranging from the smooth muscle cells of vessel walls to highly complex behaviors that are under the control of the CNS. Although often overlooked, the behavioral component, specifically ingestive behavior, is critical, especially in the response to fluid deficit, because intake is the only effective way for mammals to replace the lost fluids without medical intervention.

Angiotensin II (AngII) is a key component in this behavior. The well-known vasoconstrictive and central pressor effects of AngII help restore blood pressure, and AngII acts centrally to increase water and salt intake. These ingestive behaviors ensure the critical repletion of fluid. Moreover, the effect of AngII on drinking behavior has served as a powerful model for the diverse roles of AngII that range from blood pressure control to development [6] to an array of neurological disorders (e.g., Parkinson's and Alzheimer's diseases [7, 8]). Indeed, drugs that target AngII receptors ("ARBs") or the synthesis of AngII (e.g., ACE inhibitors) are commonly used to fight hypertension. It is notable, however, that clinical trials for these drugs show efficacy in the sense that they lower pressure, but the reduction in pressure often fails to achieve normotensive levels (for example, see [9]). Accordingly, studies of AngII on drinking behavior are not only relevant because of the importance of the behavior in the regulation of fluid homeostasis, but also because the information gathered is often applicable to the regulation of other systems by AngII.

Ingestive responses to AngII

A wealth of studies have focused on the water and saline intake stimulated by AngII (for review see [10–13]). Water intake after central injection of AngII is arguably one of the most reliable displays of effective behavioral pharmacology. The drinking response after injection of AngII is so reliable that these are commonly used to verify accurate forebrain intracerebroventricular cannula placement, even in laboratories otherwise disinterested in studying fluid intake.

Much is known about the intracellular signaling that occurs downstream of AngII receptors and the behavioral relevance of those signaling pathways (for review see [14]). As observed with other seven-transmembrane G protein-coupled receptors, stimulation of the AngII type 1 (AT₁) receptor, the receptor most closely associated with the ingestive responses to AngII, leads to a series of intracellular events that hinge upon G protein activation. The AT₁ receptor couples to G_q, and agonist binding at the receptor leads to phospholipase C (PLC) activation, causing the formation of inositol trisphosphate (IP₃) and diacylglycerol (DAG). IP₃ binds intracellular receptors to liberate stores of intracellular Ca²⁺ while DAG activates protein kinase C (PKC). In addition to this "traditional" signal transduction pathway, AT₁ receptor agonists can act independent of G proteins to stimulate mitogen-activated protein kinase (MAP kinase) family members via interactions with β -arrestin (for review, see [15]).

Our previous studies led to the development of what we refer to as the "divergent signaling hypothesis of angiotensin-induced water and saline intake" [16–19]. According to this hypothesis, AngII stimulates water intake through G protein-dependent signaling pathways, whereas saline intake is more dependent on MAP kinase-mediated signaling. In support of this hypothesis, treatment with a biased AngII receptor agonist, Sar¹,Ile⁴,Ile⁸-AngII (SII), an AngII analog that leads to phosphorylation of ERK1/2 without concomitant G protein activation [20–22], blocked water intake stimulated by AngII, but when given alone, SII increased saline intake in an AngII-like manner [18]. Moreover, pretreatment with a PKC inhibitor attenuated water intake, but not saline intake, after AngII administration, whereas pretreatment with a MAP kinase inhibitor had the opposite effect, attenuating saline intake,

but not water intake [17]. This hypothesis has found additional support from other laboratories. For instance, PEP7 was recently found to inhibit AngII-induced phosphorylation of ERK1/2 and, consistent with our hypothesis, pre-treatment with PEP7 blocks the saline intake, but not the water intake, that occurs after central injection of AngII [23].

Angiotensin II-induced desensitization

Although our initial view was that water intake stimulated by AngII was largely unaffected by MAP kinase family members, more recent studies have found a previously unappreciated role for MAP kinase family members in the control of water intake. These studies focused on the behavioral desensitization that occurs after acute repeated injections of AngII. Studies using *in vitro* [24–29] and *in vivo* [30–35] models demonstrated a tachyphylaxis (desensitization) that occurs after exposure to AngII. Our laboratory has spent several years exploring this phenomenon and we have found that, in our hands, the most consistently effective treatment regimen comprised three treatment injections in 20-min intervals, 300 ng of AngII each, followed by a test injection of 100 ng AngII [32]. This “pulsed” approach has become standard in our laboratory and has been used to learn several important features relevant to the observable desensitization.

Angiotensin II-induced desensitization: alternative explanations

The desensitization that occurs after repeated injections of AngII does not appear to be a function of motor impairment or general suppression of ingestive behaviors. We have drawn this conclusion for several reasons, including our findings that a) the pulsed treatment regimen failed to affect carbachol-induced water intake [34] and failed to affect saline intake (but still affected water intake) in a two bottle test [32]. Studies using flavor preference conditioning, performed in collaboration with Kevin Myers (Bucknell University), indicate that the decreased water intake is not a function of any negative or aversive property of the pulsed treatment itself. Indeed, after conditioning trials that paired the repeated injections with a novel flavor, rats either showed no preference, or preferred, instead of avoided, the flavor that was paired with the repeated injections of AngII [34]. Accordingly, we were able to rule out several alternative explanations for the decreased intake, in spite of the much higher cumulative dose of AngII.

In addition to ruling out general suppression of ingestive behavior, motoric deficits, and an aversive affective component of the pulsed treatments, we also tested for the possibility that elevated blood pressure was responsible for the decreased intake. This finding became important for other reasons as our line of research developed, but at the time, the critical question was if a sensitized pressor response caused the decreased intake. Indeed, central injection of AngII increases blood pressure [36], and hypertension can inhibit water intake stimulated by central AngII [37, 38]. This alternative explanation was ruled out when we found that the pressor response to AngII was not higher when AngII was given after a series of pulsed AngII injections than it was after pulsed injections of vehicle [34]. Although not statistically significant in these experiments, the pressor response actually appeared to be decreased, rather than increased, by the treatment. Low statistical power prevented us from

drawing any conclusions about the potential for desensitization of the pressor response, but provided strong evidence to rule out the contribution of sensitization of the pressor response. Accordingly, although this finding would become more interesting to us as we interpreted the results of later studies, at the time it served as an important step toward ruling out the alternative explanation that the decreased intake was a function of increased blood pressure.

Angiotensin II-induced desensitization: the role of MAP kinase (ERK1/2) and the AV3V region

Armed with the confidence that the decreased intake observed after the pulsed treatment with AngII was not due to motoric deficits, general suppressive effects, malaise, or elevated blood pressure, we conducted a series of experiments to learn more about the signaling and anatomical requirements for the observed desensitization. Two key conclusions drawn from these studies are that MAP kinase family members are critical for the observed behavioral changes, and that the anteroventral third ventricle (AV3V) region plays an important role. The former conclusion is based on a series of experiments that used a similar approach to that used when we developed the divergent signaling hypothesis mentioned earlier. Specifically, we found that pulsed injections of SII, the AngII analog described above that stimulates MAP kinase (ERK1/2) activation without concomitant G protein activity, decreased AngII-induced water intake with a similar magnitude as pulsed injections of AngII [33]. Conversely, pre-treatment with a MAP kinase inhibitor prevented the desensitization normally observed after pulsed injections of AngII [33]. Although these studies, and our conclusions based upon them, focused on the role of MAP kinase, it is entirely possible that the pathway is affected at a different level. The repeated treatment with AngII may more directly affect the receptor's ability to interact with a variety of intermediate signaling molecules, such as β -arrestin, that shift the signaling cascade toward MAP kinase activation. Whatever the primary effect of repeated AngII, the dependence on MAP kinase for the behavioral desensitization appears to be necessary because the MAP kinase inhibitor disrupted the effect.

Our conclusion that cells in the AV3V region play a critical role is based on a separate set of experiments that used AV3V microinjections. The role of the AV3V was initially explored because of its known importance in the control of fluid intake by AngII. This importance has been established using a variety of approaches, including lesions [39, 40], receptor binding [41], and studies of brain activation implied by deoxyglucose uptake [42] or Fos-immunohistochemistry [43–46]. Studies from our laboratory additionally ruled out any requirement of AngII activation in the hindbrain for the drinking response to AngII [47]. Accordingly, it seemed reasonable to hypothesize that the AV3V was a critical site for the desensitizing response to pulsed AngII, and our studies confirmed this hypothesis. Specifically, pulsed injections directly into the AV3V, using doses of AngII that failed to cause desensitization when delivered to the lateral ventricle, produced a marked reduction in the drinking response to a subsequent injection of AngII delivered either to the AV3V or to the lateral ventricle [48]. In other words, pulsed injections of AngII directly into the AV3V, presumably not directly activating other structures, were sufficient to desensitize the drinking response to a subsequent injection of AngII into the ventricle. Conversely, a single

injection of the AT₁ receptor antagonist, losartan, directly into the AV3V was sufficient to block the desensitizing effect of pulsed AngII injections into the lateral ventricle [48], thereby demonstrating the importance of AngII acting within the AV3V to cause the observed desensitization. This does not, however, suggest that the AV3V acts alone. Indeed, the changes in the behavior that are observed are likely an effect of altered signaling in several nodes of the circuit. Determining how the circuit is altered, and which alterations are critical mediators of the effect is an important avenue for future research.

Repeated injections of AngII have bivalent effects: sensitization by chronic repeated injections of AngII

Although the information presented above suggests a desensitizing effect of AngII, it appears that timing is a critical consideration because repeated exposure to AngII in a longer timeframe generates an opposite, sensitizing effect. Daily injections of AngII sensitize the pressor response to subsequent AngII [49–51]. Likewise, daily injections of AngII, repeated elevation of endogenously produced AngII, or repeated bouts of sodium or water deprivation sensitize water and saline intakes [52–59]. In recent years, our laboratory has focused on the possibility that this sensitization can be prevented using proper timing of AngII exposure.

Given the importance of the AV3V in the behavioral response, we first tested the hypothesis that repeated injection of AngII into the brain (icv) would produce changes in AT₁ receptor expression in the AV3V. Indeed, we found that a bolus injection of AngII significantly elevated AT₁ receptor mRNA in the AV3V, and also in the subfornical organ (SFO), but not in the paraventricular nucleus of the hypothalamus (PVN); however, this increase was not found when the same cumulative dose of AngII was given in three pulses of AngII [60]. Behavioral studies in our laboratory confirmed the findings of Moellenhoff *et al* [53] by showing that daily bolus injections of 10 or 40 ng of AngII caused more water intake on the fifth day of treatment than was observed on the first day of the treatment. On the other hand, when we delivered the 40 ng dose in four separate injections with 20 minutes between each (the pulsed approach used in our desensitization studies), we found that intake after the injection was the same on day 1 and day 5 [60] (Figure 2). Because the cumulative dose (40 ng) produced sensitization when given daily, and each injection in the pulsed series delivered 10 ng, which was sufficient to cause sensitization when given in a single daily injection, we conclude that the timing of the injections was the critical variable for the different intakes observed. Indeed, the data suggest that when given with the correct timing, repeated injections of AngII can prevent the sensitization that would otherwise occur with bolus injections of AngII.

An analysis of receptor-level changes that accompanied the repeated injections of AngII revealed an interesting change in receptor binding. Specifically, in the dorsal portion of the median preoptic nucleus (dMnPO; what might be considered the most dorsal part of the AV3V by some), we found that daily injections of AngII, which were found to sensitize the behavioral response, were associated with less receptor binding, and that this apparent decrease in binding did not occur when rats were given pulsed injections on each day of the experiment [60]. The direction of the effect was certainly the opposite of what we might

have expected, but the presence or absence of a difference was consistent with the behavioral observations. In other words, the treatment that caused a change in behavior (sensitization), was associated with a change in receptor binding, whereas the treatment that prevented the change in behavior (the pulsed injections) was associated with binding levels similar to controls. Although it seemed reasonable to predict that the increased behavior would be accompanied by an increase in the receptors, our findings were, nevertheless, consistent with a previous study that found less AngII-induced Fos expression in the MnPO of rats after daily injections of AngII [53]. As such, it seems that increased sensitivity to AngII appears paradoxically to accompany reduced binding and activity of cells in the MnPO.

Summary and Conclusions

Collectively, our studies demonstrate that the pulsed injection protocol makes rats less sensitive to AngII, and that the effect is not due to malaise, general suppression of behavior, motor disturbances, or an anti-dipsogenic increase in blood pressure. The effect requires MAP kinase activation, and can be produced by activation of the AT1 receptor in a way that does not stimulate G protein-mediated responses. The AV3V appears to be a critical site for the effect, and selective manipulation of the AV3V was able to produce or prevent the desensitizing effect of AngII, even when delivered to the lateral ventricle. Moreover, the increased intake and changes in receptor binding associated with daily bolus injections of AngII do not occur when the same cumulative daily dose of AngII is given in the pulsed manner typical of our studies of desensitization, suggesting that the desensitizing treatment approach can prevent the sensitization caused by AngII.

Although a great deal of work is needed before any of these findings could be considered translational or pre-clinical, these studies have the potential, remote as it may seem now, to usher in a new strategy for the treatment of hypertension. If hypertension is, indeed, related to a sensitizing effect of AngII, as proposed previously [51, 61], properly timed increases in AngII could become a useful preventative or correctional approach in subsets of hypertensive patients. Although all of the studies we have conducted to date are most accurately classified as “basic research,” it is our hope that these studies, with the help of ongoing and planned experiments, will serve as a foundation to justify considering what would be a radical shift in the approach to anti-hypertensive therapy, particularly in patients who are included in the sizable group that are resistant to treatment [5]. A shift of this magnitude would require a considerable change in thinking about the approaches to treating hypertension. Indeed, every single anti-hypertensive treatment with any relationship to AngII is designed to *reduce* angiotensinergic tone, whereas we propose that acute, properly timed *increases* in angiotensinergic tone could be effective. This complete reversal of treatment strategy is not without precedent. Indeed, a paradigm shift of similar magnitude occurred two decades ago in the treatment of congestive cardiomyopathy. Treatment of congestive cardiomyopathy initially focused on maintaining or even increasing adrenergic tone, which was thought to be the only thing keeping the patients alive. This completely changed and treatment strategies now more successfully rely on *decreasing* adrenergic signaling in the heart using cardio-selective β -blocking agents (for review, [62]). Of course we believe it is premature to think that our studies will lead to this type of a change, but we can dare to dream.

Acknowledgments

I am grateful to the SSIB organizers for the opportunity to present our work in the symposium and for the invitation to publish the proceedings in *Physiology & Behavior*.

Many of the studies described in this symposium report were conducted by Dr. Peter Vento as part of his dissertation studies. Subsequent studies were conducted by Philip Whalen and Dr. Jessica Santollo. The studies described were funded largely by the National Heart Lung and Blood Institute (HL-091911).

References Cited

1. Murphy SL, Kochanek KD, Xu J, Heron M. Deaths: Final data for 2012. *Natl Vital Stat Rep*. 2015; 63:1–117.
2. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012; 380:2095–2128. [PubMed: 23245604]
3. G. B. D. Mortality CoD, Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2015; 385:117–171. [PubMed: 25530442]
4. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012; 380:2224–2260. [PubMed: 23245609]
5. Persell SD. Prevalence of resistant hypertension in the United States, 2003–2008. *Hypertension*. 2011; 57:1076–1080. [PubMed: 21502568]
6. Hubert C, Savary K, Gasc JM, Corvol P. The hematopoietic system: a new niche for the renin-angiotensin system. *Nat Clin Pract Cardiovasc Med*. 2006; 3:80–85. [PubMed: 16446776]
7. Mertens B, Vanderheyden P, Michotte Y, Sarre S. The role of the central renin-angiotensin system in Parkinson's disease. *J Renin Angiotensin Aldosterone Syst*. 2010; 11:49–56. [PubMed: 19861346]
8. Wright JW, Harding JW. Importance of the brain Angiotensin system in Parkinson's disease. *Parkinsons Dis*. 2012; 2012:860923. [PubMed: 23213621]
9. Suzuki H, Kanno Y. Efficacy of Candesartan on Outcome in Saitama Trial G. Effects of candesartan on cardiovascular outcomes in Japanese hypertensive patients. *Hypertens Res*. 2005; 28:307–314. [PubMed: 16138560]
10. Fluharty, SJ. The neuroendocrinology of body fluid homeostasis. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, Brain, and Behavior*. Amsterdam; Boston: Academic Press; 2002. p. 525-570.
11. Daniels D, Fluharty SJ. Salt appetite: a neurohormonal viewpoint. *Physiol Behav*. 2004; 81:319–337. [PubMed: 15159174]
12. Daniels, D. Neuropeptides and Regulation of Water Intake. In: Koob, GF.; Le Moal, M.; Thompson, RF., editors. *Encyclopedia of Behavioral Neuroscience*. Oxford: Academic Press; 2010. p. 425-431.
13. Daniels, D.; Fluharty, SJ. Neuroendocrinology of Body Fluid Homeostasis. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones Brain and Behavior*. Second. San Diego: Academic Press; 2009. p. 259-288.
14. de Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T. International union of pharmacology. XXIII. The angiotensin II receptors. *Pharmacol Rev*. 2000; 52:415–472. [PubMed: 10977869]
15. Shukla AK, Xiao K, Lefkowitz RJ. Emerging paradigms of beta-arrestin-dependent seven transmembrane receptor signaling. *Trends Biochem Sci*. 2011; 36:457–469. [PubMed: 21764321]
16. Daniels D, Alan N. Epstein award: Intracellular signaling and ingestive behaviors. *Physiol Behav*. 2010; 100:496–502. [PubMed: 20346964]
17. Daniels D, Mietlicki EG, Nowak EL, Fluharty SJ. Angiotensin II stimulates water and NaCl intake through separate cell signalling pathways in rats. *Exp Physiol*. 2009; 94:130–137. [PubMed: 18723579]

18. Daniels D, Yee DK, Faulconbridge LF, Fluharty SJ. Divergent behavioral roles of angiotensin receptor intracellular signaling cascades. *Endocrinology*. 2005; 146:5552–5560. [PubMed: 16123155]
19. Daniels D, Yee DK, Fluharty SJ. Angiotensin II receptor signalling. *Exp Physiol*. 2007; 92:523–527. [PubMed: 17329311]
20. Holloway AC, Qian H, Pipolo L, Ziogas J, Miura S, Karnik S, et al. Side-chain substitutions within angiotensin II reveal different requirements for signaling, internalization, and phosphorylation of type 1A angiotensin receptors. *Mol Pharmacol*. 2002; 61:768–777. [PubMed: 11901215]
21. Miura S, Zhang J, Matsuo Y, Saku K, S SK. Activation of extracellular signal-activated kinase by angiotensin II-induced Gq-independent epidermal growth factor receptor transactivation. *Hypertens Res*. 2004; 27:765–770. [PubMed: 15785012]
22. Wei H, Ahn S, Shenoy SK, Karnik SS, Hunyady L, Luttrell LM, et al. Independent beta-arrestin 2 and G protein-mediated pathways for angiotensin II activation of extracellular signal-regulated kinases 1 and 2. *Proc Natl Acad Sci U S A*. 2003; 100:10782–10787. [PubMed: 12949261]
23. Liu J, Yosten GL, Ji H, Zhang D, Zheng W, Speth RC, et al. Selective inhibition of angiotensin receptor signaling through Erk1/2 pathway by a novel peptide. *Am J Physiol Regul Integr Comp Physiol*. 2014; 306:R619–R626. [PubMed: 24523339]
24. Thomas WG. Regulation of angiotensin II type 1 (AT1) receptor function. *Regul Pept*. 1999; 79:9–23. [PubMed: 9930579]
25. Thomas WG, Motel TJ, Kule CE, Karoor V, Baker KM. Phosphorylation of the angiotensin II (AT1A) receptor carboxyl terminus: a role in receptor endocytosis. *Mol Endocrinol*. 1998; 12:1513–1524. [PubMed: 9773975]
26. Sasamura H, Dzau VJ, Pratt RE. Desensitization of angiotensin receptor function. *Kidney Int*. 1994; 46:1499–1501. [PubMed: 7699989]
27. Gebke E, Muller AR, Jurzak M, Gerstberger R. Angiotensin II-induced calcium signalling in neurons and astrocytes of rat circumventricular organs. *Neuroscience*. 1998; 85:509–520. [PubMed: 9622248]
28. Hunyady L, Catt KJ, Clark AJ, Gaborik Z. Mechanisms and functions of AT(1) angiotensin receptor internalization. *Regul Pept*. 2000; 91:29–44. [PubMed: 10967200]
29. Guo DF, Sun YL, Hamet P, Inagami T. The angiotensin II type 1 receptor and receptor-associated proteins. *Cell Res*. 2001; 11:165–180. [PubMed: 11642401]
30. Quirk WS, Wright JW, Harding JW. Tachyphylaxis of dipsogenic activity to intracerebroventricular administration of angiotensins. *Brain Res*. 1988; 452:73–78. [PubMed: 3401750]
31. Torsoni MA, Carvalheira JB, Calejari VC, Bezerra RM, Saad MJ, Gontijo JA, et al. Angiotensin II (AngII) induces the expression of suppressor of cytokine signaling (SOCS)-3 in rat hypothalamus - a mechanism for desensitization of AngII signaling. *J Endocrinol*. 2004; 181:117–128. [PubMed: 15072572]
32. Vento PJ, Daniels D. Repeated administration of angiotensin II reduces its dipsogenic effect without affecting saline intake. *Exp Physiol*. 2010; 95:736–745. [PubMed: 20228119]
33. Vento PJ, Daniels D. Mitogen-activated protein kinase is required for the behavioural desensitization that occurs after repeated injections of angiotensin II. *Exp Physiol*. 2012; 97:1305–1314. [PubMed: 22581747]
34. Vento PJ, Myers KP, Daniels D. Investigation into the specificity of angiotensin II-induced behavioral desensitization. *Physiol Behav*. 2012; 105:1076–1081. [PubMed: 22192708]
35. Zapparoli A, Figueiredo JF, Boer PA, Rocha Gontijo JA. Impaired dipsogenic and renal response to repetitive intracerebroventricular angiotensin II (AngII) injections in rats. *J Renin Angiotensin Aldosterone Syst*. 2011; 12:161–168. [PubMed: 21393358]
36. Wright JW, Morseth SL, Abhold RH, Harding JW. Pressor action and dipsogenicity induced by angiotensin II and III in rats. *Am J Physiol*. 1985; 249:R514–R521. [PubMed: 4061676]
37. Thunhorst RL, Lewis SJ, Johnson AK. Role of arterial baroreceptor input on thirst and urinary responses to intracerebroventricular angiotensin II. *Am J Physiol*. 1993; 265:R591–R595. [PubMed: 8214151]
38. Thunhorst RL, Johnson AK. Effects of arterial pressure on drinking and urinary responses to intracerebroventricular angiotensin II. *Am J Physiol*. 1993; 264:R211–R217. [PubMed: 8430881]

39. Morris MJ, Wilson WL, Starbuck EM, Fitts DA. Forebrain circumventricular organs mediate salt appetite induced by intravenous angiotensin II in rats. *Brain Res.* 2002; 949:42–50. [PubMed: 12213298]
40. Buggy J, Johnson AK. Angiotensin-induced thirst: effects of third ventricle obstruction and periventricular ablation. *Brain Res.* 1978; 149:117–128. [PubMed: 656950]
41. Allen AM, Zhuo J, Mendelsohn FA. Localization and function of angiotensin AT1 receptors. *Am J Hypertens.* 2000; 13:31S–38S. [PubMed: 10678286]
42. Kadekaro M, Cohen S, Terrell ML, Lekan H, Gary H Jr, Eisenberg HM. Independent activation of subfornical organ and hypothalamo-neurohypophysial system during administration of angiotensin II. *Peptides.* 1989; 10:423–429. [PubMed: 2502773]
43. Rowland NE, Li BH, Fregly MJ, Smith GC. Fos induced in brain of spontaneously hypertensive rats by angiotensin II and co-localization with AT-1 receptors. *Brain Res.* 1995; 675:127–134. [PubMed: 7796121]
44. Rowland NE, Li BH, Rozelle AK, Fregly MJ, Garcia M, Smith GC. Localization of changes in immediate early genes in brain in relation to hydromineral balance: intravenous angiotensin II. *Brain Res Bull.* 1994; 33:427–436. [PubMed: 8124581]
45. Rowland NE, Li BH, Rozelle AK, Smith GC. Comparison of fos-like immunoreactivity induced in rat brain by central injection of angiotensin II and carbachol. *Am J Physiol.* 1994; 267:R792–R798. [PubMed: 8092325]
46. McKinley MJ, Badoer E, Vivas L, Oldfield BJ. Comparison of c-fos expression in the lamina terminalis of conscious rats after intravenous or intracerebroventricular angiotensin. *Brain Res Bull.* 1995; 37:131–137. [PubMed: 7606488]
47. Daniels D, Marshall A. Evaluating the potential for rostral diffusion in the cerebral ventricles using angiotensin II-induced drinking in rats. *Brain Res.* 2012; 1486:62–67. [PubMed: 23026079]
48. Vento PJ, Daniels D. The anteroventral third ventricle region is critical for the behavioral desensitization caused by repeated injections of angiotensin II. *Behav Brain Res.* 2014; 258:27–33. [PubMed: 24144549]
49. Dickinson CJ, Yu R. The progressive pressor response to angiotensin in the rabbit. *J Physiol.* 1967; 190:91–99. [PubMed: 4292200]
50. Dickinson CJ, Lawrence JR. A slowly developing pressor response to small concentrations of angiotensin. Its bearing on the pathogenesis of chronic renal hypertension. *Lancet.* 1963; 1:1354–1356. [PubMed: 14027719]
51. Xue B, Zhang Z, Johnson RF, Johnson AK. Sensitization of slow pressor angiotensin II (Ang II)-initiated hypertension: induction of sensitization by prior Ang II treatment. *Hypertension.* 2012; 59:459–466. [PubMed: 22215719]
52. Pereira DT, Menani JV, De Luca LA Jr. FURO/CAP: a protocol for sodium intake sensitization. *Physiol Behav.* 2010; 99:472–481. [PubMed: 20035777]
53. Moellenhoff E, Blume A, Culman J, Chatterjee B, Herdegen T, Lebrun CJ, et al. Effect of repetitive icv injections of ANG II on c-Fos and AT(1)-receptor expression in the rat brain. *Am J Physiol Regul Integr Comp Physiol.* 2001; 280:R1095–R1104. [PubMed: 11247832]
54. Bryant RW, Epstein AN, Fitzsimons JT, Fluharty SJ. Arousal of a specific and persistent sodium appetite in the rat with continuous intracerebroventricular infusion of angiotensin II. *J Physiol.* 1980; 301:365–382. [PubMed: 7411437]
55. Sakai RR, Fine WB, Epstein AN, Frankmann SP. Salt appetite is enhanced by one prior episode of sodium depletion in the rat. *Behav Neurosci.* 1987; 101:724–731. [PubMed: 3675851]
56. Sakai RR, Frankmann SP, Fine WB, Epstein AN. Prior episodes of sodium depletion increase the need-free sodium intake of the rat. *Behav Neurosci.* 1989; 103:186–192. [PubMed: 2923672]
57. Wirtshafter D, Covelo IR, Salija I, Stratford TR. Effects of muscimol in the nucleus accumbens shell on salt appetite and sucrose intake: a microstructural study with a comment on the sensitization of salt intake. *Behav Neurosci.* 2012; 126:699–709. [PubMed: 22889311]
58. Clark JJ, Bernstein IL. Sensitization of salt appetite is associated with increased "wanting" but not "liking" of a salt reward in the sodium-deplete rat. *Behav Neurosci.* 2006; 120:206–210. [PubMed: 16492132]

59. Pereira DT, David RB, Menani JV, De Luca LA Jr. Episodes of water deprivation enhance daily hypertonic NaCl intake in rats. *Braz J Med Biol Res.* 2002; 35:465–468. [PubMed: 11960196]
60. Santollo J, Whalen PE, Speth RC, Clark SD, Daniels D. Properly timed exposure to central ANG II prevents behavioral sensitization and changes in angiotensin receptor expression. *Am J Physiol Regul Integr Comp Physiol.* 2014; 307:R1396–R1404. [PubMed: 25354729]
61. Xue B, Zhang Z, Roncari CF, Guo F, Johnson AK. Aldosterone acting through the central nervous system sensitizes angiotensin II-induced hypertension. *Hypertension.* 2012; 60:1023–1030. [PubMed: 22949534]
62. Bristow MR. beta-adrenergic receptor blockade in chronic heart failure. *Circulation.* 2000; 101:558–569. [PubMed: 10662755]

Highlights

- Hypertension and cardiovascular disease are growing health concerns
- Improper fluid balance is an underlying factor in hypertension
- Drinking induced by angiotensin II (AngII) is an important model system
- Repeated “pulsed” injections of AngII cause rapid desensitization
- Pulsed injections appear to counteract the sensitizing effect of chronic AngII

Figure 1.

Mortality and incidence rates of hypertension. Global deaths attributable to hypertension by age and sex in the year 2010 are shown in the top panel. The histogram was produced by data from [4]. Maps of the United States showing the change in the percent of the population with self-identified hypertension are shown in the bottom panel. Maps were generated using data from the Behavioral Risk Factor Surveillance System (BRFSS) conducted by the US Centers for Disease Control and Prevention (CDC). The map shows the percent of respondents over 18 years old who report ever having been told by a doctor, nurse, or other health professional that they have high blood pressure. Women who were told they had high blood pressure only during pregnancy, and respondents who were told they had borderline hypertension, were not included. Data were retrieved from <http://www.cdc.gov/cdi/>

Figure 2.

Repeated AngII-treatment differentially influenced fluid intake. Single daily injections of either 10 or 40 ng AngII were associated with greater intake on Day 5 than was observed after the injection on Day 1. When rats were given four daily injections of 10 ng (with 20 min between each) every day for 5 days, there was no difference in water intake between days 1 and 5. *Greater than Day 1, $p < 0.05$. Figure and legend appeared originally in Santollo *et al.* [60].