IFN-γ to act as a regulator. Therefore, when compared with its general proinflammatory properties, a therapeutic window for use of IFN-γ as a regulatory cytokine, if it exists, is likely to be extremely narrow and clinically unpredictable. It is of interest to note that some of the currently approved immunomodulatory drugs for autoimmune disease may work partially through IFN-γ induction. One example is glatiramer acetate (GA), a treatment option for MS. GA is a mixture of random polymers composed of four amino acids frequently appearing in myelin basic protein, a putative autoantigen for MS. It stimulates T cells to produce both Th1 and Th2 cytokines, including IFN-γ. One of the possible regulatory mechanisms of GA may involve its ability to induce adaptive Tregs by inducing Foxp3 expression through IFN-γ production (20). By the same token, care must be taken in design of anti–IFN-γ therapy for the treatment of autoimmune disease, as such therapy may considerably interfere with the regulatory activity of endogenously produced IFN-γ. Much has yet to be learned about the molecular mechanism(s) required for IFN-γ to switch its roles at different stages of inflammation.

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1. Du, W.M. 1989. The continuity of being: Chinese visions of nature. In *Nature in Asian traditions of thought.* J.B. Callicott and R. Ames, editors. State University Press of New York. Albany, New York, USA. 73–78.
2. Mosmann, T.R., Cherwinski, H., Bond, M.W., Giedlin, M.A., and Coffman, R.L. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* **136**:2348–2357.
3. Harrington, L.E., et al. 2005. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol.* **6**:1123–1132.
4. Park, H., et al. 2005. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat. Immunol.* **6**:1133–1141.
5. Ivanov, I.I., et al. 2006. The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell.* **126**:1121–1133.
6. Rosloniec, E.F., Latham, K., and Guedez, Y.B. 2002. Paradoxical roles of IFN-gamma in models of Th1-mediated autoimmunity. *Arthritis Res.* **4**:333–336.
7. Ferber, I.A., et al. 1996. Mice with a disrupted INF-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). *J. Immunol.* **156**:5–7.
8. Vermeire, K., et al. 1997. Accelerated collagen-induced arthritis in IFN-gamma receptor-deficient mice. *J. Immunol.* **158**:5507–5513.
9. Manoury-Schwartz, B., et al. 1997. High susceptibility to collagen- induced arthritis in mice lacking IFN-gamma receptors. *J. Immunol.* **158**:5501–5506.
10. Guedez, Y.B., et al. 2001. Genetic ablation of interferon-gamma up-regulates interleukin-1beta expression and enables the elicitation of collagen-induced arthritis in a nonsusceptible mouse strain. *Arthritis Rheum.* **44**:2413–2424.
11. Guillonneau, C., et al. 2007. CD40Ig treatment results in allograft acceptance mediated by CD8+CD45RClow T cells, IFN-γ, and indoleamine 2,3-dioxygenase. *J. Clin. Invest.* **117**:1096–1106. doi:10.1172/JCI28801.
12. Wang, Z., et al. 2006. Role of IFN-γ in induction of Foxp3 and conversion of CD4+ CD25- T cells to CD4+ Tregs. *J. Clin. Invest.* **116**:2434–2441. doi:10.1172/JCI25826.
13. Wan, B., et al. 2006. Aberrant regulation of synovial T cell activation by soluble co-stimulatory molecules in rheumatoid arthritis. *J. Immunol.* **177**:8844–8850.
14. Zhu, L., et al. 2006. Synovial autoreactive T cells in rheumatoid arthritis resist IDO-mediated inhibition. *J. Immunol.* **177**:8226–8233.
15. Sawitzki, B., et al. 2005. IFN-gamma production by alloantigen-reactive regulatory T cells is important for their regulatory function in vivo. *J. Exp. Med.* **201**:1925–1935.
16. Komiyama, Y., et al. 2006. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. *J. Immunol.* **177**:566–573.
17. Aringer, M., and Smolgen, J.S. 2004. Tumour necrosis factor and other proinflammatory cytokines in systemic lupus erythematosus: a rationale for therapeutic intervention. *Lupus.* **13**:344–347.
18. Chan, W.T. 1969. *A source book in Chinese philosophy.* Princeton University Press. Princeton, New Jersey, USA. 481–488.
19. Panitch, H.S., Hirsch, R.L., Haley, A.S., and Johnson, K.P. 1987. Exacerbations of multiple sclerosis in patients treated with gamma interferon. *Lancet.* **1**:893–895.
20. Hong, J., Li, N., Zhang, X., Zheng, B., and Zhang, J.Z. 2005. Induction of CD4+CD25+ regulatory T cells by copolymer-I through activation of transcription factor Foxp3. *Proc. Natl. Acad. Sci. U. S. A.* **102**:6449–6454.

The renin-angiotensin system: it's all in your head

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Components of the renin-angiotensin system (RAS) are expressed in a number of areas in the brain involved in cardiovascular control. However, it has been difficult to link RAS actions in circumscribed brain regions to specific physiological functions. In a study appearing in this issue of the _JCI_, Sakai and associates use a combination of sophisticated transgenic techniques and stereotaxic microinjection of recombinant viral vectors to demonstrate a pivotal role in the regulation of thirst and salt appetite of angiotensin II generated in the subfornical organ in the brain (see the related article beginning on page 1088).

The renin-angiotensin system (RAS) is a key regulator of blood pressure and fluid homeostasis (1). The main effector molecule of the RAS, angiotensin II, is produced from the substrate angiotensinogen through sequential enzymatic cleavages by renin and angiotensin converting enzyme. In the classic view of the RAS (Figure 1A), derived from analysis of its components in the circulation, the liver is the primary source of angiotensinogen. In the circulating system, the amount of renin in the plasma is a key rate-limiting step determining the overall RAS activity. The kidney is the major source of circulating renin where its secretion is triggered by reduced pressures in the afferent arteriole, reduced sodium delivery to the macula densa, and activation of β-sympathetic neurons. Thus, the activity of the circulating RAS is precisely calibrated by signals from the kidney linked to blood pressure and intake of salt and water. Angiotensin II generated within the circulation is then transported throughout the body, triggering a range of physiological responses.

It has become clear that this simplified view of the RAS (Figure 1A) cannot fully explain the physiological complexity of the system in health and disease. For example,

A Classic RAS

B Brain RAS
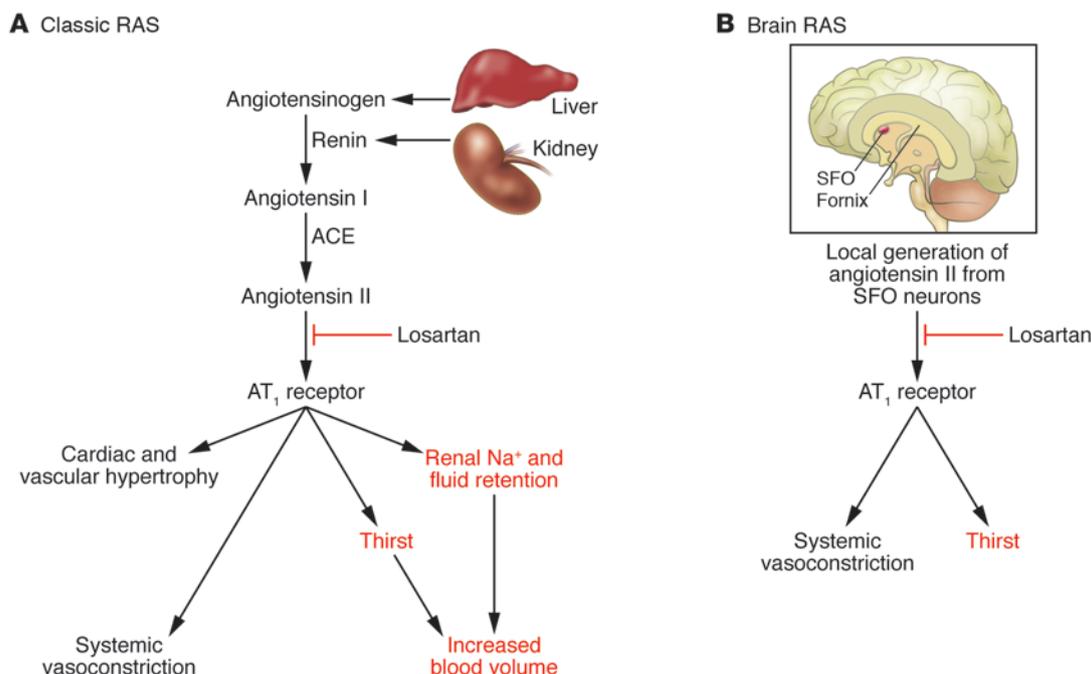
Figure 1
RAS signaling. (**A**) Classic RAS. Angiotensinogen is excreted from the liver and converted to angiotensin II through sequential cleavage by the enzymes renin and angiotensin-converting enzyme (ACE). Circulating angiotensin II activates AT$_1$ receptors in numerous target tissues, resulting in responses such as increased water and sodium reabsorption, cell proliferation, and changes in vascular tone. In this system, renin synthesis and excretion by the kidney determine the levels of circulating angiotensin II and therefore its downstream effects. (**B**) Brain RAS. In this issue of the *JCI*, Sakai and colleagues (4) demonstrate that production of angiotensin II in the SFO from locally derived angiotensinogen and renin has profound effects on thirst and salt appetite. If local synthesis of renin is controlled independently of kidney-derived renin, this would allow for distinct regulation of the physiological responses to angiotensin II within the brain.

in the broad population of patients with hypertension, diabetes, and cardiovascular disease, pharmacological antagonists of the RAS lower blood pressure and prevent end-organ damage even in the absence of overt elevation of plasma renin levels (2). Moreover, various components of the RAS are synthesized in tissues throughout the body where their expression may be subject to local control (3). Accordingly, it has been suggested that compartmentalized RASs may operate within individual organ systems with some degree of autonomy to influence regional responses. The studies from the Sigmund and Davisson laboratories suggest that the brain may possess such a local RAS (Figure 1B), which may affect drinking behavior (see the study by Sakai et al. in this issue; ref. 4) and systemic pressor responses (5).

RAS on the brain
The capacity of the CNS to respond directly to angiotensin II was demonstrated in experiments more than 2 decades ago, wherein angiotensin II was injected into cerebral ventricles or specific brain nuclei

and it subsequently elicited potent cardio-vascular and dipsogenic (thirst-provoking) responses (6, 7). Expression of virtually all of the components of the RAS has since been verified in various regions and cell lineages in the brain. Based on these findings, it was suggested that angiotensin II generated locally in the brain might function as a putative neurotransmitter in neurons involved in cardiovascular regulation (8). Within the brain, the subfornical organ (SFO) is a major site of RAS activity that has also been implicated as an important cardiovascular control center. Injection of angiotensin II into the SFO activates its neurons and elicits potent systemic vaso-constriction (7, 9) (Figure 1B). While angio-tensinogen and angiotensin receptors are highly expressed within the SFO (10–14), it lies outside of the blood-brain barrier and therefore is also potentially subject to modulation by components of the RAS in the circulation.

Many of the general features of the brain RAS have been apparent for some time. Until recently, however, it has been difficult to develop a precise understand-

ing of its contributions to physiological homeostasis in the intact organism. This was due to a number of experimental barriers, including gaps in expression patterns of RAS components between regions and difficulties in precisely manipulating the brain RAS in vivo. The development of techniques for transgenesis and gene targeting advanced the field, but the lack of promoters to drive expression in specific brain regions remains a significant limitation. In the current study by Sakai and associates (4), along with another recent publication by the same group (5), trans-genic mouse lines expressing RAS genes have been utilized in combination with microinjection of recombinant viral vectors to establish a critical contribution of angiotensin II generation within the SFO to the control of thirst and systemic vaso-pressor responses (Figure 1B).

A physiological role for angiotensin II production in the SFO
Angiotensinogen is broadly expressed in glial cells throughout the brain, while its expression in neurons is restricted to spe-

cific populations, including those in the SFO. Whether renin is expressed in the brain has been difficult to document and has been a matter of some debate. Proponents have suggested that problems verifying expression are due to relatively low levels of renin expression in the CNS. Recent work using sensitive transgenic reporter lines provided new evidence supporting expression of renin in a number of brain regions including the SFO, primarily in the neurons of the SFO (15, 16).

In order to assess the putative physiological significance of neuronal renin, Sakai et al. (4) used transgenic mice expressing human *renin* under the control of the neuron-specific *synapsin I* promoter (SR mice). These SR mice were intercrossed with a second line of transgenic mice, expressing the human *angiotensinogen* gene controlled by its endogenous promoter, to generate double-transgenic mice (SRA mice). Between humans and mice, there is strong species specificity for the enzymatic cleavage of angiotensinogen by renin, so that expression of either transgene alone does not affect angiotensin II levels and therefore causes no phenotype. However, SRA double-transgenic mice exhibited dramatic increases of water and salt intake. This was accompanied by a robust increase in angiotensin II immunoreactivity localized primarily to the SFO (Figure 1B). This phenotype was abolished by icv infusion of the Ang II receptor, type 1 (AT$_1$) blocker losartan, consistent with previous studies showing that dipsogenic responses to angiotensin II are mediated by AT$_{1B}$ receptors in the CNS (17).

The SFO lies outside the blood-brain barrier, and it was therefore conceivable that transgene-derived human angiotensinogen in the circulation might have contributed to the increase in local angiotensin II that was observed in the SRA mice (4). In order to determine the pertinent source of *angiotensinogen* and to test its physiological role, Sakai and associates took advantage of a second transgenic line, in which the principal coding exon of human angiotensinogen transgene is flanked by loxP sites. In the presence of Cre recombinase, the exon would be excised, producing a nonfunctional *angiotensinogen* gene. When crossed with SR mice, the double-transgenic SRA^{flox} animals displayed a phenotype that was identical to that of the SRA mice: increased water and salt intake associated with exaggerated angiotensin II levels in the SFO. Using stereotaxic microinjection, adeno-

viruses expressing Cre recombinase were injected into the SFO of the SRA^{flox} animals. This caused a significant decrease in drinking and reduced angiotensin II immunoreactivity in the SFO.

Taken together, these studies suggest that angiotensin II, arising from angiotensinogen and renin expressed in SFO neurons, plays a critical role in RAS-dependent drinking and salt appetite (Figure 1B). Although the SFO is outside of the blood-brain barrier, angiotensinogen derived from the circulation apparently cannot substitute for its local expression in the SFO neurons. Since SRA mice lack circulating human renin, it is not clear whether neuronal expression of renin would be required to drive this response in the presence of normal levels of renin in plasma. Nevertheless, this experiment clearly illustrates the capacity for local generation of angiotensin II in the SFO to control drinking behavior.

In separate experiments using a similar system, this group also showed that expression of angiotensinogen in the SFO is critical for centrally-mediated vasoconstrictor responses (5). In this case, Cre-mediated deletion of the floxed human *angiotensinogen* transgene in SRA^{flox} mice abrogated the vasoconstrictor and bradycardiac effects caused by infusion of human renin into the CNS. These findings highlight the importance of local expression of angiotensinogen in the control of cardiovascular functions by the SFO. Moreover, they suggest a capacity for interaction between the circulating and local RASs in modulating this systemic vascular response (Figure 1).

Many RASs: how do they fit together?

The work from Sakai et al. (4) and Sinnayah et al. (5) provides indisputable evidence for nonredundant contributions of a brain RAS to control drinking behavior and systemic cardiovascular responses. Thus, local expression of RAS components in individual tissues can have essential actions that may operate independently of the RAS in the circulation. These findings raise a number of interesting questions for future investigation. Do the systemic and local systems have overlapping function and regulation? In particular, are the production of renin and angiotensinogen regulated in the brain, and if so, what are the pertinent regulatory pathways? One would imagine that any system controlling renin levels in the CNS must be very

different from the mechanisms regulating renin secretion in the kidney. This regulatory system would likely be triggered by distinct local and environmental cues, perhaps linked to changes in osmolality and/or regional neural circuits. Furthermore, since β-adrenergic activity is a major determinant of renin secretion by the kidney, the connection between the brain RAS and the sympathetic nervous system suggests a possible mechanism for direct interaction between RASs in the CNS and the circulation.

The current studies (4, 5) focus on normal physiological and homeostatic responses. What happens during disease? Can augmentation or dysregulation of local RAS in organs such as the brain contribute to disease pathogenesis in hypertension or other cardiovascular disorders? Global pharmacological inhibition or traditional gene targeting cannot differentiate systemic from local actions of the RAS. Thus, as illustrated by the studies by Sakai and colleagues (4, 5), creative use of techniques allowing for cell- and tissue-specific manipulation of gene expression in vivo will be essential for answering these questions.

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1. Peach, M.J. 1977. Renin-angiotensin system: biochemistry and mechanisms of action. *Physiol. Rev.* **57**:313–370.
2. Brunner, H.R., et al. 1979. Oral angiotensin-converting enzyme inhibitor in long-term treatment of hypertensive patients. *Ann. Intern. Med.* **90**:19–23.
3. Dzau, V.J., Ellison, K.E., Brody, T., Ingelfinger, J., and Pratt, R.E. 1987. A comparative study of the distributions of renin and angiotensinogen messenger ribonucleic acids in rat and mouse tissues. *Endocrinology.* **120**:2334–2338.
4. Sakai, K., et al. 2007. Local production of angiotensin II in the subfornical organ causes elevated drinking. *J. Clin. Invest.* **117**:1088–1095. doi:10.1172/JCI31242.
5. Sinnayah, P., et al. 2006. Genetic ablation of angiotensinogen in the subfornical organ of the brain prevents the central angiotensinergic pressor response. *Circ. Res.* **99**:1125–1131.
6. Lind, R.W., Swanson, L.W., and Ganten, D. 1985. Organization of angiotensin II immunoreactive cells and fibers in the rat central nervous system. An immunohistochemical study. *Neuroendocrinology.* **40**:2–24.
7. Mangiapane, M.L., and Simpson, J.B. 1980. Subfornical organ: forebrain site of pressor and dipsogenic action of angiotensin II. *Am. J. Physiol.* **239**:R382–R389.
8. Brosnihan, K.B., Diz, D.I., Schiavone, M.T., Averill, D.B., and Ferrario, C.M. 1987. Approaches to establishing angiotensin II as a neurotransmitter. In *Brain peptides and catecholamines in cardiovascular*

regulation. J.P. Buckley and C.M. Ferrario, editors. Raven Press. New York, New York, USA. 313–328.

9. Phillips, M.I., and Felix, D. 1976. Specific angiotensin II receptive neurons in the cat subfornical organ. *Brain Res.* **109**:531–540.

10. Davisson, R.L., et al. 1998. The brain renin-angiotensin system contributes to the hypertension in mice containing both the human renin and human angiotensinogen transgenes. *Circ. Res.* **83**:1047–1058.

11. Yang, G., Gray, T.S., Sigmund, C.D., and Cassell, M.D. 1999. The angiotensinogen gene is expressed in both astrocytes and neurons in murine central nervous system. *Brain Res.* **817**:123–131.

12. Chen, Y., and Morris, M. 2001. Differentiation of brain angiotensin type 1a and 1b receptor mRNAs: a specific effect of dehydration. *Hypertension.* **37**:692–697.

13. Lenkei, Z., Corvol, P., and Llorens-Cortes, C. 1995. The angiotensin receptor subtype AT1A predominates in rat forebrain areas involved in blood pressure, body fluid homeostasis and neuroendocrine control. *Brain Res. Mol. Brain Res.* **30**:53–60.

14. Morris, M., et al. 1999. Neuroendocrine effects of dehydration in mice lacking the angiotensin AT1a receptor. *Hypertension.* **33**:482–486.

15. Lavoie, J.L., Cassell, M.D., Gross, K.W., and Sigmund, C.D. 2004. Adjacent expression of renin and angiotensinogen in the rostral ventrolateral medulla using a dual-reporter transgenic model. *Hypertension.* **43**:1116–1119.

16. Lavoie, J.L., Cassell, M.D., Gross, K.W., and Sigmund, C.D. 2004. Localization of renin expressing cells in the brain, by use of a REN-eGFP transgenic model. *Physiol. Genomics.* **16**:240–246.

17. Davisson, R.L., Oliverio, M.I., Coffman, T.M., and Sigmund, C.D. 2000. Divergent functions of angiotensin II receptor isoforms in the brain. *J. Clin. Invest.* **106**:103–106.