Central Renin–Angiotensin System Activation and Inflammation Induced by High-Fat Diet Sensitize Angiotensin II–Elicited Hypertension

Baojian Xue, Robert L. Thunhorst, Yang Yu, Fang Guo, Terry G. Beltz, Robert B. Felder, Alan Kim Johnson

Abstract—Obesity has been shown to promote renin–angiotensin system activity and inflammation in the brain and to be accompanied by increased sympathetic activity and blood pressure. Our previous studies demonstrated that administration of a subpressor dose of angiotensin (Ang) II sensitizes subsequent Ang II–elicited hypertension. The present study tested whether high-fat diet (HFD) feeding also sensitizes the Ang II–elicited hypertensive response and whether HFD-induced sensitization is mediated by an increase in renin–angiotensin system activity and inflammatory mechanisms in the brain. HFD did not increase baseline blood pressure, but enhanced the hypertensive response to Ang II compared with a normal-fat diet. The sensitization produced by the HFD was abolished by concomitant central infusions of either a tumor necrosis factor-α synthesis inhibitor, pentoxifylline, an Ang II type 1 receptor blocker, irbesartan, or an inhibitor of microglial activation, minocycline. Furthermore, central pretreatment with tumor necrosis factor-α mimicked the sensitizing action of a central subpressor dose of Ang II, whereas central pentoxifylline or minocycline abolished this Ang II–induced sensitization. Real-time quantitative reverse transcription–polymerase chain reaction analysis of lamina terminalis tissue indicated that HFD feeding, central tumor necrosis factor-α, or a central subpressor dose of Ang II upregulated mRNA expression of several components of the renin–angiotensin system and proinflammatory cytokines, whereas inhibition of Ang II type 1 receptor and of inflammation reversed these changes. The results suggest that HFD-induced sensitization of Ang II–elicited hypertension is mediated by upregulation of the brain renin–angiotensin system and of central proinflammatory cytokines. (Hypertension. 2016;67:163-170. DOI: 10.1161/HYPERTENSIONAHA.115.06263) ● Online Data Supplement

Key Words: angiotensin II ■ blood pressure ■ high-fat diet ■ proinflammatory cytokine ■ sensitization

Converging lines of evidence indicate that hypertension is characterized by increased activity of the renin–angiotensin system (RAS) and elevated levels of proinflammatory cytokines (PICs). The interactions and synergism between the RAS and PICs have been studied in both the periphery and the central nervous system (CNS). We and others have demonstrated that systemic angiotensin (Ang) II administration elicits an increase in blood pressure (BP) that is accompanied by an upregulation of mRNA expression of PICs in the brain.1,2 Central injections of PICs, such as tumor necrosis factor (TNF)-α or interleukin (IL)-1β, significantly elevate BP, renal sympathetic nerve activity (RSNA), and brain angiotensin type 1 receptor (AT1-R) and angiotensin-converting enzyme (ACE1) mRNA.3,4 Conversely, central inhibition of TNF-α synthesis or blockade of AT1-R significantly reduced the hypertensive effect and diminished the upregulation of mRNA for RAS components and PICs produced by systemic Ang II.1,5

Diet-induced obesity in both humans and rodents is associated with an increased prevalence of hypertension.6 Head et al recently reported that 3 weeks of high-fat diet (HFD) feeding led to increased mean arterial pressure (MAP), heart rate (HR), and RSNA in rabbits. Autonomic ganglion blockade completely abolished the increase in BP, suggesting that this model of obesity-induced hypertension is neurogenic.7–9 Obese animals have been shown to have increased RAS activity and elevated levels of Ang II that upregulates AT1-R in a variety of tissues, including the brain.10,11

Recent studies also demonstrate that exposure to a HFD induces hypothalamic as well as peripheral inflammation,12 and one day of exposure to a HFD (60%) has been shown to be sufficient to increase hypothalamic cytokine expression.13 Interestingly, although animals with diet-induced obesity can...
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restore body weight (BW), insulin levels, and leptin sensitivity to normal after being switched to normal-fat diet (NFD), BP, RSNA, and the activity of central RAS and PICs remain high. These results indicate that obesity itself may induce hypothalamic inflammation and sensitization of the brain to circulating sympathoexcitatory factors, such as those from the RAS, and these initiate increased BP.

Forebrain structures along with the lamina terminalis (LT), including the subfornical organ (SFO), median preoptic nucleus, and organum vasculosum, play important roles in the long-term regulation of BP, body fluid, and energy homeostasis. Our previous work demonstrated that the BP response that manifests as hypertension can be sensitized by treating male rats with mild physiological or dietary challenges at a point earlier in the animal’s lifetime. Initial studies found that pretreatment with subpressor doses of either Ang II or aldosterone can upregulate the RAS in the LT and sensitize the hypertensive response to a pressor dose of Ang II and to high dietary salt. Given that brain RAS activation and inflammation are common features of both obesity and hypertension, such results prompted us to hypothesize that exposure to high dietary salt may also sensitize the Ang II–elicited hypertensive response and that the brain RAS and TNF-α signaling may contribute to that response.

Methods

Experimental Protocol

Male Sprague–Dawley rats (10- to 12-weeks old, Harlan, n=131) were used. Rats were prepared with a lateral ventricular cannula, osmotic minipumps for intracerebroventricular (ICV) and subcutaneous drug infusion, and with telemetry probes for continuous BP monitoring, as previously described. The ICV doses of agents used to induce sensitization or to block AT1-R, TNF-α synthesis, or microglial activation were chosen on the basis of published in vivo studies.

All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by The University of Iowa Animal Care and Use Committee.

Effects of HFD on Ang II–Induced Hypertension and Molecular Events in the LT

BP was monitored continuously by telemetry in rats that were fed either NFD (10% calories from lard, 3.85 kcal/g, D12450J, Research Diet, NJ) or HFD (60% calories from lard, 5.24 kcal/g, D12492, Research Diet, NJ) for 3 weeks (induction period [I]), followed by NFD for 2 weeks (expression period [E]). During I, rats received ICV vehicle (V), the TNF-α synthesis inhibitor pentoxifylline (PTX, 10 μg/h), the AT1-R blocker irbesartan (Irbe,125 μg/d), or the inhibitor of microglial activation minocycline (Mino, 5 μg/h). These inhibitors were delivered by osmotic pumps (model 2004, 0.25 μL/h; Alzet) for 1 week. To ensure that any exogenous Ang II and antagonists were metabolized, the rats then rested for 1 week (D). During this period, a second pump (model 2002, 0.5 μL/h; Alzet) was implanted to deliver a slow pressor dose of Ang II (120 ng/kg/min) for 2 weeks (E). Thus, the primary study groups (n=6/group) were (1) I-ICV V+E-Ang II, (2) I-ICV Ang II+E-Ang II, (3) I-ICV TNF-α+E-Ang II, (4) I-ICV Ang II/PDX+E-Ang II, and (5) I-ICV Ang II/Mino+E-Ang II.

Five additional groups (n=5/group) underwent identical treatment during I (I-ICV V, I-ICV TNF-α, I-ICV Ang II, I-ICV Ang II/PTX, and I-ICV Ang II/Mino) but were euthanized at the end of the D period to collect LT for analysis of mRNA expression.

Results

HFD-Induced Sensitization of Ang II Hypertension

Baseline values for MAP (105.6±2.3 mmHg) and HR (346.9±5.5 bpm) were comparable before and after application of HFD or ICV infusion of PTX, Irbe, or Mino alone in all groups of rats (Figure S1 in the online-only Data Supplement). However, over the course of E, Ang II induced a greater increase in MAP in the rats that received the HFD (Δ39.4±3.7 mmHg, 2-way ANOVA: effect of HFD, F(1, 30)=8.69, P=0.0061; effect of Ang II, F(2, 30)=93.81, P<0.0001; Figure 1A and 1B) as compared with the rats pretreated with NFD (Δ25.4±2.8 mmHg). This augmentation of the pressor effect induced by Ang II was blocked by concurrent ICV infusions of either PTX (Δ25.6±3.5 mmHg, 2-way ANOVA: effect of ICV PTX, F(1, 30)=6.53, P=0.0159; effect of Ang II, F(2, 30)=247.6, P<0.0001), Irbe (Δ24.4±4.4 mmHg, 2-way ANOVA: effect of ICV Irbe, F(1, 30)=9.31, P=0.0047; effect of Ang II, F(2, 30)=117.6, P<0.0001), or Mino (Δ27.9±2.4 mmHg, 2-way ANOVA: effect of ICV Mino, F(1, 30)=7.31, P=0.0112; effect of Ang II, F(2, 30)=204.7, P<0.0001) along with the HFD pretreatment (P<0.05). Systemic Ang II infusions produced slight, comparable decreases in HR in all groups (Figure S2).

The hexamethonium-induced decreases in MAP were comparable in all groups before HFD treatment (averaged =21.6±0.6 mmHg; Figure 1C). HFD alone or HFD combined with central inhibition of TNF-α synthesis, AT1-R, or microglial activation also had no effect on the decrease in MAP produced by acute ganglionic blockade (averaged =23.8±1.4 mmHg; Figure 1C). However, after 14 days of Ang II infusion, acute hexamethonium injection resulted in significant
decreases in MAP in both NFD- and HFD-treated rats (P<0.05 versus before infusion of Ang II). Notably, HFD-pretreated rats exhibited an enhanced decrease in MAP (HFD −56.1±2.3 mm Hg versus NFD −38.7±2.5 mm Hg, P<0.05, Figure 1C), which was attenuated by central inhibition of TNF-α synthesis (−33.8±3.2 mm Hg, P<0.05), AT1-R (−37.8±4.4 mm Hg, P<0.05), or microglial activation (−35.2±3.5 mm Hg, P<0.05).

Figure 1. Augmented pressor effects induced by Ang II during the expression (E) period in rats after pretreatment with high-fat diet (HFD) during the induction (I) period. A, This effect was attenuated by central inhibition of tumor necrosis factor (TNF)-α synthesis, Ang II type 1 receptor (AT1-R), or microglia activation. B, The changes in mean arterial pressure (MAP) after infusion of angiotensin (Ang) II during (E) in all groups. C, The decreases in MAP in response to ganglionic blockade with hexamethonium at baseline, after 3 weeks of diet treatment, and on day 14 after infusion of Ang II in all groups. E-Ang II indicates peripheral treatment with a pressor dose of Ang II during E; E-saline, peripheral treatment with saline during E; I-ICV V, central treatment with vehicle during I; I-HFD, pretreatment with high-fat diet during I; I-HFD+ICV Irbe, pretreatment with HFD plus central treatment with AT1-R blocker irbesartan (Irbe) during I; I-HFD+ICV Mino, pretreatment with HFD plus central treatment with inhibitor of microglial activation minocycline (Mino) during I; I-HFD+ICV PTX, pretreatment with HFD plus central treatment with TNF-α synthesis inhibitor pentoxifylline (PTX) during I; and I-NFD, pretreatment with normal fat diet during I; *significant difference vs baseline or after diet treatment; #significant difference vs I-NFD+ICV V+E-Ang II and other groups fed with HFD plus central blocker treatment during I).

Caloric Intake, Feed Efficiency, and BW During HFD and Systemic Ang II

During I, all groups of rats fed the HFD had slight increases in caloric intake (71.2±3.5 kcal/d, P>0.05; Figure 2A), but exhibited significantly higher feed efficiency (38.0±3.2 mg BW/kcal, P<0.05; Figure 2B) when compared with the group of NFD-fed rats (64.5±4.1 kcal/d and 27.8±2.8 mg BW/kcal). Accordingly, 3 weeks of HFD feeding resulted in a significant increase in BW (HFD, 58.2±5.2 g versus NFD 38.5±4.3 g, P<0.05; Figure 2C). During E, with all groups receiving the NFD and the Ang II infusion, caloric intake was comparable in rats that had been induced with NFD or HFD (P>0.05), and the rates of BW gain were significantly reduced in all groups (P<0.05, Figure 2C).

Effect of ICV PTX, Irbe, or Mino on HFD-Induced mRNA Expression of RAS and Inflammatory Elements in the LT

In LT tissue collected at the end of HFD feeding, HFD induced a significant increase in the mRNA expression of the inflammatory elements (ie, TNF-α, IL-6, the microglial marker CD11b) and some (ie, renin, AT1-R, ACE1), but not all, RAS components in the LT when compared with controls (P<0.05).
The expression of angiotensinogen (AGT), mineralocorticoid receptor, NADPH oxidase 2, and IL-1β in the LT was not affected by HFD (P>0.05, Figure 3A and 3B).

Central infusion of PTX, Irbe, or Mino normalized the increased mRNA expression of renin, TNF-α, IL-6, and CD11b produced by HFD (P<0.05, Figure 3A and 3B). Among the RAS components whose expression was upregulated by HFD, the increased AT1-R expression was attenuated by ICV PTX, the elevated expression of AGT and ACE1 were inhibited by ICV Irbe, whereas the increased expression of AGT and AT1-R was attenuated by ICV Mino (P<0.05; Figure 3A and 3B). Message for mineralocorticoid receptor, NADPH oxidase 2, and IL-1β remained unchanged.

**ICV TNF-α and Low-Dose Ang II–Induced Sensitization of Ang II Hypertension**

To confirm the direct sensitizing actions of central TNF-α and its mediation of central Ang II–induced sensitization, TNF-α, or a supressor dose of Ang II combined with either a TNF-α synthesis inhibitor or an inhibitor of microglial activation was infused ICV for 1 week during I. These treatments had no effect on MAP during I (Figure 4A). The ICV infusion of TNF-α mimicked the ICV supressor dose of Ang II–induced augmentation of the pressor effect produced by a subsequent pressor dose of Ang II during E (TNF-α, Δ44.5±3.4 mmHg, 2-way ANOVA: effect of ICV TNF-α, F(1, 30)=6.51, P=0.016; effect of Ang II, F(2, 30)=168.3, P<0.0001; Ang II, Δ42.8±5.3 mmHg, 2-way ANOVA: effect of ICV Ang II, F(1, 30)=6.65, P=0.015; effect of Ang II, F(2, 30)=120.0, P<0.0001) as compared with that of ICV saline–treated rats (Δ25.7±4.1 mmHg; Figure 4A and 4B). This augmentation of the hypertensive response induced by the pressor dose of Ang II was abolished by concurrent ICV infusion of the TNF-α synthesis inhibitor PTX or by the inhibitor of microglial activation Mino along with the ICV supressor dose of Ang II administrated during I (PTX Δ28.2±1.9 mmHg, 2-way ANOVA: effect of ICV PTX, F(1, 30)=5.04, P=0.0324; effect of Ang II, F(2, 30)=137.7, P<0.0001; Mino Δ21.7±3.5 mmHg, 2-way ANOVA: effect of ICV Mino, F(1, 30)=4.97, P=0.0334; effect of Ang II, F(2, 30)=141.6, P<0.0001; Figure 4A and 4B). Systemic Ang II infusions produced slight, but comparable decreases in HR in all groups (Figure S3).

**Effect of ICV PTX or Mino on Central Suppressor Dose of Ang II–Induced mRNA Expression of RAS Components, PICs, and Microglial Marker in the LT**

In LT tissue collected at the end of D period, the ICV supressor dose of Ang II given during I produced a significant increase in the mRNA expression of renin, AGT, AT1-R, ACE1, mineralocorticoid receptor, NADPH oxidase 2, TNF-α, IL-6, and CD11b (P<0.05), but had no effect on the mRNA expression of IL-1β when compared with controls. The ICV infusion of TNF-α during I similarly elicited most of the increases in the mRNA expression induced by Ang II except the mRNA expression of AGT, mineralocorticoid receptor, TNF-α, but had a significant potentiating effect on the
mRNA expression of IL-1β. Concurrent central infusion of PTX or Mino along with a sensitizing dose of Ang II blocked the enhanced increase in mRNA expression in most cases (P<0.05) with the exception of AT1-R (Figure 5A and 5B).

**Discussion**

Obesity-related hypertension is a major risk factor for the development of cardiovascular diseases. These studies provide evidence that a short-term exposure to HFD activates the RAS and PICs in the LT and sensitizes the pressor actions of Ang II. A similar sensitization of the Ang II–induced hypertensive response can be produced by ICV administration of TNF-α or a subpressor dose of Ang II. These observations indicate that activation of the RAS and PICs and the interaction between these 2 systems are involved in the process of sensitization. The sensitizing action of HFD is also associated with activation of microglia, which express AT1-R and PIC receptors and are a potential source of PICs. Taken together, these results suggest that eating a HFD for a short time can induce remarkable changes in CNS function that increase the vulnerability to hypertension.

Overproduction of Ang II or excessive activation of the brain RAS contributes to the genesis of cardiovascular diseases, including hypertension. Ang II can act as a potent proinflammatory agent and stimulate the production of PICs, such as TNF-α, IL-6, and IL-1β in the brain that augment hypertensive responses. The possible cellular sources of brain PICs include CD11b+ microglia and neurons. A recent study demonstrated that inflammation and microglial activation within the hypothalamic paraventricular nucleus are associated with elevated BP and augmented sympathetic activity induced by Ang II infusion. Furthermore, ICV TNF-α injection elevates BP and sympathetic activity in a dose-dependent manner. PICs act within the SFO to upregulate the expression of components of the RAS and mediators of inflammation that elicit a sympathoexcitatory response mediated by the RAS. Central inhibition of TNF-α reverses alterations in RAS components and attenuates Ang II–induced hypertension. Moreover, both Ang II and TNF-α can activate NADPH oxidase, leading to enhanced oxidative stress and decreased bioavailability of NO that combine to contribute to increases in SNA and BP. These studies confirm that the brain RAS and PICs can mutually facilitate each other’s expression in the CNS, as well as the sympathoexcitatory and pressor effects that generate hypertension. Previous studies from our laboratory have shown that subpressor doses of Ang II and aldosterone given during I produced upregulation of components of the brain RAS, resulting in a sensitized hypertensive response to a subsequently administered, pressor dose of Ang II. The present study extends our previous work by showing that central pretreatment with TNF-α mimicked the sensitizing actions of Ang II, upregulating mRNA expression of the RAS components, PICs, microglial marker, and NADPH oxidase in the LT and augmenting the hypertensive response to a pressor dose of Ang II. Inhibition of either TNF-α synthesis or microglia activation abolished these changes. These results indicate that Ang II upregulation of PICs and microglial activation mediates Ang II–induced sensitization and suggest that low-grade central inflammation induced by different classes of stimuli can sensitize the brain to be predisposed to the expression of frank hypertension.

The RAS and PICs have both been implicated in the regulation of energy balance and obesity-induced hypertension. Not only does adipose tissue have a local RAS, but serum levels of major components of the RAS (renin, AGT, ACE) are elevated in obesity. TNF-α is commonly considered as one of the initiators of the proinflammatory cascade, which can induce the production of other cytokines. Inhibition of TNF-α during inflammatory events abolishes many of the ensuing responses, including increased IL-1β and IL-6. TNF-α levels are increased in obesity and serve as a marker for obesity. Recent studies have revealed that HFD feeding increases hypothalamic PIC expression, including IL-1β, IL-6, and TNF-α, and several components of the RAS, such as AT1-R. The increased gene expression for the RAS and for PICs is accompanied by increased microglial activation in the hypothalamus, including the arcuate nucleus, SFO, and hypothalamic paraventricular nucleus. Some of these responses were reversed on deletion of AT1a specifically within the hypothalamic paraventricular nucleus.

It has been shown that systemic RAS and TNF-α, which are both increased in obesity, play a role in promoting energy storage, whereas these 2 systems act within the CNS to increase energy expenditure. The difference between the central and peripheral effects of the RAS and TNF-α suggests the presence of a negative feedback mechanism that is activated when peripheral RAS activity and TNF-α levels are high and gain access to the brain. However, this putative central negative feedback pathway for energy balance triggers the cardiovascular consequences of activation of the RAS.
and PICs with resultant elevations of SNA and BP. Consistent with the activating effect of obesity on the brain, we found in the present study that the HFD feeding significantly elicited increases in mRNA expression of several RAS components, PICs, and a microglial marker in the LT. These changes might reflect a mechanism for sensitizing the brain cardiovascular nuclei and enhance their reactivity, which is evident by the enhanced hypertensive response to the subsequent Ang II infusion in the HFD-fed rats. Furthermore, central inhibition of AT1-R and inflammation during HFD feeding abolished the HFD-induced sensitizing effect on hypertension. Combined with the findings that a low dose of TNFα or Ang II administered centrally directly sensitizes Ang II–induced hypertension, these observations suggest that the HFD-induced upregulation of the RAS and PICs in the CNS participates in the sensitization process. The capacity of the RAS or PICs to mutually upregulate their expression may be indicative of actions within central sensitization-related positive feedforward systems, which can accelerate the onset and rate of development of hypertension.

It should be noted that many previous studies have used long-term HFD feeding (8 weeks or more longer time) to produce obesity and elevated levels of RAS components and of PICs (eg, TNF-α) in both brain and peripheral tissues. However, one recent study showed that in rats predisposed to diet-induced obesity, the expression of proinflammatory biomarkers was increased in the mediodorsal hypothalamus within 24 h of HFD onset, suggesting that hypothalamic inflammation occurs before obesity onset. This raises questions as to what factor(s) or through which pathway HFD activates the CNS RAS and inflammation so that the brain is sensitized to circulating hypertensive agents in the initial stages of ovornutrition before the advent of increased systemic inflammation and RAS activation.

It has been shown that the hypothalamic structures residing inside blood–brain barrier, including the arcuate nucleus, hypothalamic paraventricular nucleus, supraoptic nucleus, and lateral hypothalamus, are major sites for the regulation of autonomic and energy processes. The LT, including the SFO, a sensory circumventricular organ lacking the normal blood–brain barrier, has also been documented as a CNS structure involved in both cardiovascular regulation and energy homeostasis. The SFO sends efferent projections to communicate with these hypothalamic nuclei inside blood–brain barrier. In the present study, we found that 3 weeks of HFD feeding significantly increased the mRNA expression of the RAS and PICs in the LT. This result suggests that the components of the LT may first sense factors produced by HFD feeding and respond to the changes in the energy homeostasis by producing increased activation of the RAS and PICs in the cardiovascular neural network without apparent changes in the BP and sympathetic activity. Further study is needed to investigate the hypothalamic nuclei involving the HFD-induced sensitization process, as well as looking more specifically at the pathway between the SFO and the hypothalamic nuclei involved in processing signals associated with the RAS and PICs that are initiated in the course of developing obesity.

Recently, a series of studies from Head et al demonstrated in rabbits that 3 weeks of HFD feeding rapidly produced increased MAP, HR, and RSNA. This obesity-induced hypertension could be blocked by a central leptin antagonist after 3 weeks of HFD, which is a time when BW, visceral white adipose tissue, and plasma leptin levels were markedly elevated. They also found that the hypertension and high RSNA persisted long after stopping HFD and the normalization of circulating levels of leptin. These findings raise the possibility that HFD produces factors in addition to leptin that contribute to the increases in BP and SNA. It is likely that these other factors are the RAS components and PICs. Results of the current study showing that 3 weeks of HFD feeding significantly increased BW and the mRNA expression of the RAS and PICs in the LT point to these as additional important mediating factors. Combining the results of the Head et al studies with the current findings, it can be hypothesized that either local or systemic RAS components and PICs, probably stimulated by the HFD itself or by leptin, are the critical mediators of HFD-induced sensitization of hypertension.

Because mRNA gene expression results do not always reflect protein expression or activity levels, one limitation of the present study is that we did not determine activity and protein levels of the RAS components and PICs as the basis of the changes in the mRNA levels after HFD treatment. Therefore, further studies to analyze the protein levels in cardiovascular nuclei and circulating concentrations of the RAS and PICs after short-term HFD may uncover mechanisms through which the HFD modulates the brain nuclei sensitivity that participate in the pathophysiology of obesity-related hypertension. Another limitation is that the TNF-α synthesis inhibitor PTX and the inhibitor of microglial activation Mino used in the present study are not selective and affect multiple biochemical targets. However, using both PTX and Mino produced results that converge to be highly suggestive that inflammation and related factors are involved in mediating the sensitizing effects of HFD on the hypertensive response. The use of more selective inhibitors in the future studies should lend additional support to this interpretation of our current findings.

**Perspectives**

Clinical and experimental data indicate important interactions between obesity and hypertension. Activation of both the RAS and PICs in the CNS play a critical role in the development of obesity, hypertension, or both. The present findings indicate that similar to the sensitizing effects of subpressor doses of Ang II or aldosterone, sensitization by HFD feeding is associated with maintained changes in the expression of pressor components of the brain RAS and PICs. These observations provide novel evidence and insight for the actions of RAS and of PICs on LT structures to mediate cardiovascular sensitizing effects of metabolic factors, even though these factors do not induce immediate abnormalities in the cardiovascular function. One implication of these and our demonstrations of sensitization and maintained neuroplasticity is that challenges to homeostasis can act to reprogram the neural network controlling BP to generate an enhanced pressor response when a hypertensinogenic stimulus is either sustained or encountered at a later time.
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Disclosures

None.

References


Sources of Funding

High-Fat Diet Sensitizes Ang II Hypertension

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**Novelty and Significance**

**What Is New?**

• These studies demonstrate that short-term exposure to high-fat diet sensitizes the hypertensive response to angiotensin II. Central inhibition either of proinflammatory cytokine synthesis or of renin–angiotensin system activity abolishes high-fat diet–elicited sensitization of angiotensin II hypertension.

**What Is Relevant?**

• The demonstration of high-fat diet–facilitating effect on the expression of the central renin–angiotensin system and proinflammatory cytokines, as well as the interaction between these 2 systems, indicates that central nervous system renin–angiotensin system and proinflammatory cytokines are likely to play an important role in the pathogenesis and progression of obesity-related hypertension.

**Summary**

The study indicates that high-fat diet acts on the brain to sensitize the hypertensive response to angiotensin II and that sensitization is associated with maintained altered expression of renin–angiotensin system and proinflammatory cytokines within components of a forebrain cardiovascular control network.
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Central renin-angiotensin system activation and inflammation induced by high fat diet sensitized angiotensin II hypertension

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Methods

Physiological Studies
Under Ketamine-xylazine anesthesia, rats were chronically instrumented with telemetry probes (TA11PA-C40; DSI) placed in the femoral artery for continuous monitoring of mean arterial pressure (MAP) and heart rate (HR). Beginning seven days after recovery from surgery, MAP and HR data collection was initiated. To study the sensitizing effect of high fat diet (HFD, 60% calories from lard, 5.24 kcal/g, D12492, Research Diet, NJ) on Ang II-induced hypertension, the rats were fed with HFD or normal fat diet (NFD, 10% calories from lard, 3.85 kcal/g, D12450J, Research Diet, NJ) for three weeks. These treatments were combined with administration of agents through a brain lateral ventricle cannula (the coordinates 1.0 mm caudal, 1.5 mm lateral to bregma, and 4.5 mm below the skull surface) connected to an osmotic pump containing vehicle (artificial cerebrospinal fluid for PTX and Mino or 0.84% NaHCO3 for Irbe) or inhibitors of TNF-α, AT1-R or microglia. The the HFD was replaced by NFD for two weeks and a second osmotic pump containing saline or Ang II was implanted on the back of rats under isoflurane anesthesia. Food consumption, water intake and body weight were measured once a week. Total food consumption, water intake and gain of body weight were averaged by daily. Daily calorie intake was calculated: average daily food consumption x diet energy density. Feed efficiency, the ability to convert caloric intake into BW, was determined by: mean BW gain (mg) / total caloric intake (kcal).

To study the direct sensitizing action of central TNF-α or a subpressor dose of Ang II during the Induction period, brain cannulas were connected to an osmotic pump containing these agents combined with the inhibitor of TNF-α or microglia. A second pump containing the pressor dose of Ang II was also implanted at the beginning of the Expression period. In this set of experiments, all rats were fed normal rat chow (7013 NIH-31 modified rat diet) ad libitum.

Evaluation of BP responses to autonomic blockade
The autonomic contribution to increased BP was assessed by administering the ganglionic blocker hexamethonium (30 mg/kg, ip). Ganglionic blockade was repeated three times, first during the baseline period, second after HFD pretreatment and third after 14 days of Ang II infusion. On the day of the ganglionic blockade experiments, BP was recorded for 20 min both before and after hexamethonium injection. After hexamethonium injection, the largest decrease in BP occurred within 5 min. This nadir (2-3 min) was recorded as the maximum fall in BP.

Measurement of mRNA Expression in the LT
Total RNA was isolated from LT using Trizol method (Invitrogen) and treated with DNase I (Invitrogen). RNA integrity was checked by gel electrophoresis. Total RNA was reverse transcribed using random hexamers following the manufacturer’s instructions (Applied Biosystems). Real time PCR was conducted using 200-300 ng of cDNA and 500 nM of each primer in a 20 μl reaction with iQ SYBR Green Supermix (Bio-Rad). Amplification cycles were conducted at 95°C for 3 min, followed by 40 cycles of 95°C for 15 s and annealing/extension at 60°C for 30 s. Reactions were performed in duplicate and analyzed using a C1000 thermocycler system (Bio-Rad). Samples that did not yield homogenous melt curves were excluded. Changes in mRNA expression levels were normalized to GAPDH levels and calculated using the ΔΔCt method. Results are expressed as relative fold change, mean of fold change ± SE. Primers were purchased from Integrated DNA Technologies (Coralville, IA). The sequences of the primers are shown in Table S1.
Table S1: Primer Sequences for Real Time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Product size(bp)</th>
</tr>
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<tbody>
<tr>
<td>GAPDH</td>
<td>TGACTCTACCCACGGAAGTTCAA</td>
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AGT, angiotensinogen; MR, mineralocorticoid receptor; AT-R, angiotensin receptor; ACE1, angiotensin converting enzyme 1; NOX2, NADPH oxidase 2; IL-6, interleukin-6; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; CD11b, cluster of differentiation molecule 11b.

Data Analysis

Baseline MAP and HR data were collected for 5 days and then for 28-35 consecutive days. MAP and HR are presented as mean daily values averaged from daytime and nighttime measurements. Difference scores for MAP and HR were calculated for each animal based on the mean of the 5-day baseline subtracted from the mean of the final 5 days of treatment. Two-way ANOVAs for the experimental groups (1. among NFD group, HFD groups and HFD groups with central blockers; 2. among groups with central vehicle, TNF-α, subpressor dose of Ang II alone or combined with antagonists) were then conducted on the means of calculated difference scores. After establishing a significant ANOVA, post-hoc analyses were performed with Tukey multiple comparison tests between pairs of mean change scores. One-way ANOVAs were used to analyze the differences in metabolic parameters, BP decreases induced by acute hexamethonium injection and in mRNA expression of brain RAS components and PICs in all groups. After establishing a significant ANOVA, post-hoc analyses were performed with Tukey multiple comparison tests. All data are expressed as means ± SE. Statistical significance was set at \( P < 0.05 \).
**Fig. S1**

Figure S1 shows no change in mean arterial pressure (MAP) after icv sole infusion of TNF-α synthesis inhibitor PTX, AT1-R blocker Irbe or microglia inhibitor Mino during Induction (I) and Expression (E) periods in all groups.

**Fig. S2**

Figure S2. Daily heart rate (HR) before and during normal fat diet (NFD) or high fat diet (HFD) feeding and systemic infusion of angiotensin II (Ang II) in icv vehicle, PTX, Irbe or Mino treated rats. I-NFD = pretreatment with normal fat diet during I; I-HFD = pretreatment with high fat diet during I; icv V = central treatment with vehicle during I; I-HFD+icv PTX = pretreatment with HFD plus central treatment with TNF-α synthesis inhibitor pentoxifylline during I; I-HFD+icv Irbe = pretreatment with HFD plus central treatment with AT1-R blocker irbesartan during I; I-HFD+icv Mino = pretreatment with HFD plus central treatment with microglia.
inhibitor minocycline during I; E-saline = peripheral treatment with saline during E; E-Ang II = peripheral treatment with a pressor dose of Ang II during E.

**Fig. S3**

![Graph showing heart rate (HR) during icv vehicle, PTX, Irbe or Mino in Induction (I) period and during systemic infusion of angiotensin II (Ang II) in Expression (E) period.](image)

**Figure S3.** Daily heart rate (HR) during icv vehicle, PTX, Irbe or Mino in Induction (I) period and during systemic infusion of angiotensin II (Ang II) in Expression (E) period. icv V = central treatment with vehicle during I; icv TNF-α = central treatment with TNF-α during I; icv Ang II = central treatment with subpressor dose of Ang II during I; I-icv Ang II/PTX = central concurrent treatment with subpressor dose of Ang II and TNF-α synthesis inhibitor pentoxifylline during I; I-icv Ang II/Mino = central concurrent treatment with subpressor dose of Ang II and microglia inhibitor minocycline during I; E-Ang II = peripheral treatment with a pressor dose of Ang II during E.