Subfornical Organ Mediates Sympathetic and Hemodynamic Responses to Blood-Borne Proinflammatory Cytokines

Shun-Guang Wei, Zhi-Hua Zhang, Terry G. Beltz, Yang Yu, Alan Kim Johnson, Robert B. Felder

Abstract—Proinflammatory cytokines play an important role in regulating autonomic and cardiovascular function in hypertension and heart failure. Peripherally administered proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), act on the brain to increase blood pressure, heart rate, and sympathetic nerve activity. These molecules are too large to penetrate the blood–brain barrier, and so the mechanisms by which they elicit these responses remain unknown. We tested the hypothesis that the subfornical organ (SFO), a forebrain circumventricular organ that lacks a blood–brain barrier, plays a major role in mediating the sympathetic and hemodynamic responses to circulating proinflammatory cytokines. Intracarotid artery injection of TNF-α (200 ng) or IL-1β (200 ng) dramatically increased mean blood pressure, heart rate, and renal sympathetic nerve activity in rats with sham lesions of the SFO (SFO-s). These excitatory responses to intracarotid artery TNF-α and IL-1β were significantly attenuated in SFO-lesioned (SFO-x) rats. Similarly, the increases in mean blood pressure, heart rate, and renal sympathetic nerve activity in response to intraventricular injections of TNF-α (500 ng) or IL-1β (500 ng) in SFO-s rats were significantly reduced in the SFO-x rats. Immunofluorescent staining revealed a dense distribution of the p55 TNF-α receptor and the IL-1 receptor accessory protein, a subunit of the IL-1 receptor, in the SFO. These data suggest that SFO is a predominant site in the brain at which circulating proinflammatory cytokines act to elicit cardiovascular and sympathetic responses. (Hypertension. 2013;62:118-125.) ● Online Data Supplement

Key Words: cytokine receptors ● proinflammatory cytokines ● subfornical organ ● sympathetic drive

Inflammation has been implicated in the development of a variety of cardiac, cerebrovascular, and metabolic diseases, such as heart failure, hypertension, atherosclerosis, stroke, obesity, and diabetes mellitus.1-5 Levels of inflammatory mediators are augmented in the circulation and tissues in these pathophysiological conditions. Growing evidence indicates that systemic inflammation contributes to the progression of these diseases by activating the sympathetic nervous system,6,7 but the mechanisms remain unclear.

Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) are 2 representative members of the proinflammatory cytokine family that act on the brain to increase blood pressure (BP), heart rate (HR), and renal sympathetic nerve activity (RSNA).8,9 How these molecules that are lipophobic and too large to cross the blood–brain barrier (BBB) activate the sympathetic nervous system remains a mystery. TNF-α and IL-1β receptors are widely distributed in the cerebral microvasculature and other elements of the BBB, including the choroid plexus and circumventricular organs (CVOs) that lack a BBB.10,11 There is a strong support for the hypothesis that circulating cytokines activate their receptors on endothelial and perivascular cells of the brain microvasculature to induce cyclooxygenase-2 (COX-2) activity and the production of prostaglandin E2 (PGE2), which crosses the BBB and can elicit sympathetic responses.12 In vitro brain slice work has demonstrated that IL-1β activates neurons in the subfornical organ (SFO),13 a forebrain CVO. The possibility that circulating proinflammatory cytokines might increase sympathetic drive by activating their receptors on cellular elements of CVOs has not previously been explored.

The present study examined the potential role of the SFO as a link between blood-borne proinflammatory cytokines and central mechanisms driving sympathetic excitation. The SFO has direct and indirect connections to the hypothalamic paraventricular nucleus (PVN),14,15 a key cardiovascular and autonomic brain region that contains presympathetic and neuroendocrine neurons. We chose to study the SFO because of its recognized role in the pathogenesis of heart failure,16 hypertension,17,18 and the febrile response.19

Methods

Animals

Experiments were performed on adult male Sprague-Dawley rats (300–350 g), purchased from Harlan Sprague Dawley (Indianapolis, IN) and housed two per cage in a temperature- and humidity-controlled environment with a 12-hour light-dark cycle. Rats were given free access to food and water. The rats were anesthetized with ketamine (100 mg/kg, i.m.) and xylazine (10 mg/kg, i.m.) and sacrificed by decapitation. The brains were removed, and the SFO and PVN were dissected and stored at -80°C for later assay.

Received March 15, 2013; first decision April 7, 2013; revision accepted April 14, 2013.

From the Department of Internal Medicine, University of Iowa Roy J. and Lucille A. Carver College of Medicine, Iowa City, IA (S.-G.W., Z.-H.Z., Y.Y., R.B.F.); Department of Psychology, University of Iowa, Iowa City, IA (T.G.B., A.K.J.); and Veterans Affairs Medical Center, Iowa City, IA (R.B.F.).

The online-only Data Supplement is available with this article at http://hyper.ahajournals.orglookup/suppl/doi:10.1161/HYPERTENSIONAHA.113.01404/-/DC1.

Correspondence to Robert B. Felder, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, IA 52242. E-mail robert-felder@uiowa.edu

© 2013 American Heart Association, Inc.

Hypertension is available at http://hyper.ahajournals.org

DOI: 10.1161/HYPERTENSIONAHA.113.01404
IN). Animals were housed in Animal Care Facility at the University of Iowa and fed rat chow ad libitum. All experimental procedures were approved by the University of Iowa Institutional Animal Care and Use Committee. The experimental protocols were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Drug Administration**

Intracarotid artery (ICA) injections of TNF-α and IL-1β were made via a rostrally directed polyethylene (PE)-20 cannula inserted into the left common carotid artery, with the tip placed in the bifurcation. ICA injections are thought to target primarily the forebrain in rats. Intravenous (IV) injections of TNF-α and IL-1β were performed via a PE-50 cannula placed in the left femoral vein. The IV injections allowed TNF-α and IL-1β to access receptors in the periphery and all regions of the brain. TNF-α and IL-1β were purchased from Fitzgerald (Acton, MA) and Millipore (Billerica, MA), respectively, and dissolved in 0.9% saline. Both drugs were administered as a bolus injection (200 ng in 4 μL ICA; 500 ng in 10 μL IV) followed by a 15-μL saline flush. Doses were based on a previous publication.1

**Experimental Protocols**

**Hemodynamic and Sympathetic Effects of TNF-α and IL-1β**

Rats that had undergone SFO lesions (SFO-x; n=22) or a sham procedure (SFO-s; n=22) to access receptors in the periphery and all regions of the brain. TNF-α and IL-1β were purchased from Fitzgerald (Acton, MA) and Millipore (Billerica, MA), respectively, and dissolved in 0.9% saline. Both drugs were administered as a bolus injection (200 ng in 4 μL ICA; 500 ng in 10 μL IV) followed by a 15-μL saline flush. Doses were based on a previous publication.1

**Specific Materials and Methods**

Please see the online-only Data Supplement.

**Results**

**Hemodynamic and Sympathetic Effects of Blood-Borne Cytokines in SFO-s and SFO-x Rats**

**ICA Administration**

ICA injections of TNF-α (n=6; Figure 1A and 1C) and IL-1β (n=6; Figure 2A and 2C) induced similar substantial and long-lasting increases in BP, HR, and RSNA in SFO-s rats. Both began within 10 to 20 minutes of the ICA injection. The peak increase in MBP (TNF-α: 21.8±4.1 mm Hg; IL-1β: 22.9±3.1 mm Hg) occurred at 1 hour and was sustained at a higher than baseline level for the remainder of the 4- to 5-hour recording period. The peak increases in HR (TNF-α: 101.8±11.3 bpm; IL-1β: 82.4±8.4 bpm) and RSNA (TNF-α: 114.5±12.2% change; IL-1β: 99.3±12.8% change) occurred 2 to 3 hours after the ICA injections and lasted for 4 to 5 hours and even longer in most cases.

In the SFO-x rats, ICA injections of TNF-α (n=6; Figure 1B and 1C) and IL-1β (n=6; Figure 2B and 2C) induced smaller, delayed responses in BP, HR, and RSNA. There were no significant changes in MBP, HR, or RSNA within the first 2 hours after the injections, and the peak responses of BP (TNF-α: 6.9±2.3 mm Hg; IL-1β: 8.5±2.0 mm Hg), HR (TNF-α: 46.8±8.9 bpm; IL-1β: 41.5±7.6 bpm), and RSNA (TNF-α: 50.4±10.5% changes; IL-1β: 41.3±7.3% changes) occurred 3 to 4 hours after the injections.

ICA injections of an equal volume of vehicle (0.9% saline) had no effects on MBP, HR, and RSNA in SFO-s (n=4) or SFO-x (n=4) rats (Figure S1 in the online-only Data Supplement). The baseline levels of MBP (100.4±3.2 mm Hg), HR (326±8 bpm), and integrated RSNA (10.8±3.7 nV) in SFO-s rats did not differ significantly from the baseline levels of MBP (98.6±3.1 mm Hg), HR (328±9 bpm), and integrated RSNA (10.4±3.4 nV) in SFO-x rats.

**IV Administration**

Similar to ICA injections, IV injection of either TNF-α (n=5; Figure 3A and 3C) or IL-1β (n=5; Figure 4A and 4C) markedly augmented BP, HR, and RSNA in SFO-s rats. The responses to TNF-α and IL-1β began within 10 to 20 minutes of the IV injection. The peak increase in MBP (TNF-α: 18.4±2.4 mm Hg; IL-1β: 18.0±3.2 mm Hg) occurred 1 hour, and MBP was sustained at a higher than baseline level for the remainder of the 4- to 5-hour recording period. The peak HR (TNF-α: 80.6±10.0 bpm; IL-1β: 71.3±8.3 bpm) and RSNA (TNF-α: 89.2±9.3% change; IL-1β: 85.1±10.8% change) responses occurred 2 to 3 hours after the injection and lasted for 4 to 5 hours and even longer in most cases.

In the SFO-x rats, the increases in MBP, HR, and RSNA to IV TNF-α (n=5; Figure 3B and 3C) or IL-1β (n=5; Figure 4B and 4C) were substantially blunted. However, the early responses (first 2 hours) were attenuated rather than completely blocked, as the responses to the ICA injection had been. The peak MBP (TNF-α: 8.3±2.1 mm Hg; IL-1β: 7.1±2.4 mm Hg) responses occurred at 1 to 1.5 hours after the IV injection. The peak HR (TNF-α: 40.0±7.7 bpm; IL-1β: 37.3±7.9 bpm) and RSNA (TNF-α: 45.8±9.6% change; IL-1β: 45.1±7.8% change) responses occurred 2 to 3 hours after the IV injection.

The baseline levels of MBP, HR, and RSNA were similar to those in the ICA study group. IV injections of an equal volume of vehicle (0.9% saline, n=4) had no effects on MBP, HR, and RSNA in SFO-s or SFO-x rats (data not shown).

**Histological Assessment of the SFO Lesions**

Twenty-two of 28 animals in which SFO lesions were made had ≥80% ablation of the SFO and constituted the SFO-x group. Representative images of the SFO region from SFO-s and SFO-x rats are shown in Figure S2. The excitatory responses induced by ICA and IV TNF-α or IL-1β remained intact in the 6 animals with incomplete SFO lesions (Figure S3).
Figure 1. Representative tracings (A and B) and grouped data (C) showing effects of intracarotid artery (ICA) injection of tumor necrosis factor-α (TNF-α; 200 ng) on blood pressure (BP; mm Hg), heart rate (HR; bpm), and renal sympathetic nerve activity (RSNA), windowed (spikes/s) and integrated (mV), in sham subfornical organ-lesioned (SFO-s) and SFO-lesioned (SFO-x) rats. Arrows indicate the initiation of the injections. MBP indicates mean blood pressure; and ΔRSNA (%), percent change from baseline in integrated RSNA. All values are expressed as the mean±SE. *P<0.05 compared with baseline in either SFO-s or SFO-x. †P<0.05 compared with SFO-s. Scale bar, 20 minutes.

Figure 2. Representative tracings (A and B) and grouped data (C) showing effects of intracarotid artery (ICA) injection of interleukin-1β (IL-1β; 200 ng) on blood pressure (BP; mm Hg), heart rate (HR; bpm), and renal sympathetic nerve activity (RSNA), windowed (spikes/s) and integrated (mV), in sham subfornical organ-lesioned (SFO-s) and SFO-lesioned (SFO-x) rats. Arrows indicate the initiation of the injections. MBP indicates mean blood pressure; and ΔRSNA (%), percent change from baseline in integrated RSNA. All values are expressed as the mean±SE. *P<0.05 compared with baseline in either SFO-s or SFO-x. †P<0.05 compared with SFO-s. Scale bar, 20 minutes.
Figure 3. Representative tracings (A and B) and grouped data (C) showing effects of IV injection of tumor necrosis factor-α (TNF-α; 500 ng) on blood pressure (BP; mmHg), heart rate (HR; bpm), and renal sympathetic nerve activity (RSNA), windowed (spikes/s) and integrated (mV), in sham subfornical organ-lesioned (SFO-s) and SFO-lesioned (SFO-x) rats. Arrows indicate the initiation of the injections. MBP indicates mean blood pressure; and ΔRSNA (%), percent change from baseline in integrated RSNA. All values are expressed as the mean±SE. *P<0.05 compared with baseline in either SFO-s or SFO-x. †P<0.05 compared with SFO-s. Scale bar, 20 minutes.

Figure 4. Representative tracings (A and B) and grouped data (C) showing effects of IV injection of interleukin-1β (IL-1β; 500 ng) on blood pressure (BP; mmHg), heart rate (HR; bpm), and renal sympathetic nerve activity (RSNA), windowed (spikes/s) and integrated (mV), in sham subfornical organ-lesioned (SFO-s) and SFO-lesioned (SFO-x) rats. Arrows indicate the initiation of the injections. MBP indicates mean blood pressure; and ΔRSNA (%), percent change from baseline in integrated RSNA. All values are expressed as the mean±SE. *P<0.05 compared with baseline in either SFO-s or SFO-x. †P<0.05 compared with SFO-s. Scale bar, 20 minutes.
Cytokine Receptors in the SFO and PVN of Normal Rats

Immunofluorescent studies revealed intense evenly distributed expression of TNFR1 immunoreactivity in the SFO (Figure 5A; n=6) of normal rats. Confocal immunofluorescent images indicated that TNFR1 was expressed by neurons, astrocytes, and unlabeled cellular elements in the SFO (Figure 5A). There was no significant expression of TNFR1 in the PVN in these normal rats (Figure S4).

IL-1RAcP was also intensely expressed in the SFO (Figure 5B; n=6). Like TNFR1, IL-1RAcP was evenly expressed throughout the SFO by neurons, astrocytes, and unlabeled cellular elements (Figure 5B). Expression of IL-1RAcP in the PVN was sparse in these normal rats and found only in the magnocellular subdivision of the PVN (Figure S4).

Discussion

The present study suggests an important role for the SFO in mediating the sympathetic and hemodynamic responses to circulating proinflammatory cytokines. The major findings are as follows: (1) peripheral injections of TNF-α and IL-1β induce pronounced increases in MBP, HR, and RSNA that are largely dependent on an intact SFO; and (2) receptors for TNF-α and IL-1β are abundantly expressed by neurons, astrocytes, and other cellular elements of the SFO. These findings in normal rats suggest that the SFO may be a primary interface between peripheral inflammation and central nervous system mechanisms driving sympathetic activity in chronic inflammatory conditions like hypertension and heart failure.

We used 2 strategies to determine the role of the SFO in cytokine-induced sympathetic excitation. ICA injections of TNF-α and IL-1β, which preferentially target the forebrain region, allowed an assessment of central nervous system-mediated effects, while minimizing any potential influences of peripheral cytokine receptors. The ICA dose, given intravenously, had very little effect. In this protocol, the SFO lesion prevented cytokine-induced cardiovascular and sympathetic responses in the first 100 minutes, although a small increase in all variables was observed thereafter. These results confirmed an important role for the SFO in mediating the effects of cytokines reaching the forebrain. We used IV injections of TNF-α and IL-1β to assess the role of the SFO in the response to cytokines in the systemic circulation, where they also have access to peripheral cytokine receptors (eg, in tissues innervated by vagal afferents) and cytokine receptors in other brain regions in which they are known to exert their central effects. In this protocol, which more closely resembles pathophysiological conditions, the SFO lesion also substantially reduced the cardiovascular and sympathetic responses.

Several mechanisms have been invoked to explain the central influences of circulating cytokines (Figure 6). These include induction of COX-2/PGE2, in cells of the BBB, activation of cells in the CVOs that are exposed to circulating cytokines reaching the forebrain. We used IV injections of TNF-α and IL-1β to assess the role of the SFO in the response to cytokines in the systemic circulation, where they also have access to peripheral cytokine receptors (eg, in tissues innervated by vagal afferents) and cytokine receptors in other brain regions in which they are known to exert their central effects. In this protocol, which more closely resembles pathophysiological conditions, the SFO lesion also substantially reduced the cardiovascular and sympathetic responses.

Several mechanisms have been invoked to explain the central influences of circulating cytokines (Figure 6). These include induction of COX-2/PGE2, in cells of the BBB, activation of cells in the CVOs that are exposed to circulating cytokines reaching the forebrain. We used IV injections of TNF-α and IL-1β to assess the role of the SFO in the response to cytokines in the systemic circulation, where they also have access to peripheral cytokine receptors (eg, in tissues innervated by vagal afferents) and cytokine receptors in other brain regions in which they are known to exert their central effects. In this protocol, which more closely resembles pathophysiological conditions, the SFO lesion also substantially reduced the cardiovascular and sympathetic responses.

Several mechanisms have been invoked to explain the central influences of circulating cytokines (Figure 6). These include induction of COX-2/PGE2, in cells of the BBB, activation of cells in the CVOs that are exposed to circulating cytokines reaching the forebrain. We used IV injections of TNF-α and IL-1β to assess the role of the SFO in the response to cytokines in the systemic circulation, where they also have access to peripheral cytokine receptors (eg, in tissues innervated by vagal afferents) and cytokine receptors in other brain regions in which they are known to exert their central effects. In this protocol, which more closely resembles pathophysiological conditions, the SFO lesion also substantially reduced the cardiovascular and sympathetic responses.

Several mechanisms have been invoked to explain the central influences of circulating cytokines (Figure 6). These include induction of COX-2/PGE2, in cells of the BBB, activation of cells in the CVOs that are exposed to circulating cytokines reaching the forebrain. We used IV injections of TNF-α and IL-1β to assess the role of the SFO in the response to cytokines in the systemic circulation, where they also have access to peripheral cytokine receptors (eg, in tissues innervated by vagal afferents) and cytokine receptors in other brain regions in which they are known to exert their central effects. In this protocol, which more closely resembles pathophysiological conditions, the SFO lesion also substantially reduced the cardiovascular and sympathetic responses.
cytokines,\textsuperscript{11} active transport of cytokines across the BBB,\textsuperscript{22} and activation of vagal afferent fibers.\textsuperscript{23,24} We have previously demonstrated that cytokine induction of COX-2 in perivascular cells plays a role in the sympathetic excitation in heart failure.\textsuperscript{25} Others have shown that cytokine induction of COX2/PGE\textsubscript{2} activates the hypothalamic–pituitary–adrenal axis, a response that apparently arises from the brain stem and is not affected by interrupting the descending projections to PVN from the forebrain CVOs.\textsuperscript{26} In contrast, SFO lesions can prevent the cytokine-induced febrile response.\textsuperscript{19} The present study suggests that the SFO is a predominant site in the brain at which circulating proinflammatory cytokines act to elicit excitatory cardiovascular and sympathetic responses. One or more of the other putative mechanisms indicated in Figure 6 may also contribute to the responses to circulating cytokines.

An obvious question that arises is, how do cytokines act on the SFO to elicit a sympato-excitatory response? SFO-dependent cytokine-induced sympathetic and hemodynamic responses are characterized by a delayed onset (10–20 minutes after ICA or IV injection) and a prolonged duration (>4 hours). These features suggest the induction of cellular mechanisms leading to increased neuronal excitability, rather than direct synaptic activation. The SFO is a particularly rich environment for cytokine-induced molecular signaling, with TNF-\(\alpha\) and IL-1\(\beta\) receptors expressed on multiple cell types in a nucleus exposed to the circulation. Astrocytes, microglia, perivascular macrophages, and endothelial cells all express cytokine receptors. Astrocytes produce chemokines\textsuperscript{26} and angiotensinogen,\textsuperscript{27} the precursor of angiotensin II (Ang II); microglia produce a variety of inflammatory mediators, including proinflammatory cytokines, chemokines, COX-2/PGE\textsubscript{2}, and reactive oxygen species\textsuperscript{28,29}; endothelial and perivascular cells produce COX-2 and PGE\textsubscript{2}. All these non-neuronal mechanisms may contribute to the overall excitability of SFO neurons without directly activating them. How exactly cytokine receptors on the SFO neurons themselves contribute remains uncertain; in an in vitro brain slice preparation, SFO neurons responded to IL-1\(\beta\) within seconds to minutes, but the mechanism was not determined.\textsuperscript{13}

The concept of the SFO as a sensor of peripheral inflammation and orchestrator of the central cardiovascular and sympathetic response is consistent with its known functions. The SFO has long been known as the primary central nervous system sensor of circulating Ang II, communicating its message to brain centers\textsuperscript{30} that regulate BP, sympathetic outflow, drinking behavior, and neuroendocrine release of vasopressin, oxytocin, and adrenocorticotrophic hormone.\textsuperscript{31–33} Destruction of the SFO attenuates the development of hypertension in several humorally-driven experimental models\textsuperscript{17,18,34} and the progression of heart failure after myocardial infarction.\textsuperscript{16,35} More recently, oxidative stress in the SFO induced by a slow-pressor dose of Ang II has been shown to drive immune-mediated cardiovascular dysfunction in hypertension.\textsuperscript{36} It is of note in this regard that proinflammatory cytokines also induce oxidative stress in brain tissue, independently\textsuperscript{37} and perhaps also by upregulating the activity of the brain renin-angiotensin system (RAS).\textsuperscript{38} The present results raise the possibility of a positive feedback loop in conditions like hypertension and heart failure, whereby circulating products of peripheral immune activation promote oxidative stress in SFO, amplifying the peripheral immune response and perhaps the effects of circulating Ang II. The interaction of proinflammatory cytokines with the systemic and brain RAS at the SFO level is an important area for future research in hypertension and heart failure.

Previous studies have emphasized the sympatho-excitatory role of proinflammatory cytokines in the PVN in heart failure\textsuperscript{39} and hypertension.\textsuperscript{40} It may be presumed that the cardiovascular and sympathetic responses to cytokine activation of the SFO depend on activation of presympathetic and perhaps neuroendocrine neurons downstream in the PVN.

Notably, in normal rats in the present study, TNFR1 were sparse in the PVN compared with their dense representation in the SFO. Similarly, IL-1\(\beta\) receptors were expressed only sparsely and mostly in the magnocellular subdivision of PVN that is associated with vasopressin release. These results are consistent with previous immunohistochemical studies in normal rats\textsuperscript{11,41} and with in vitro data from normal rats, suggesting

![Figure 6. The schematic illustrates putative pathways by which circulating cytokines may influence sympathetic activity. All except the vagal afferent pathway are potentially excitatory. BBB indicates blood–brain barrier; COX-2, cyclooxygenase-2; CVLM, caudal ventrolateral medulla; CVOs, circumventricular organs; IL-1\(\beta\), interleukin-1\(\beta\); NTS, nucleus tractus solitarius; OVLT, organum vasculosum of the lamina terminalis; PGE\textsubscript{2}, prostaglandin E\textsubscript{2}; PVN, paraventricular nucleus of the hypothalamus; RVLM, rostral ventrolateral medulla; SFO, subfornical organ; and TNF-\(\alpha\), tumor necrosis factor-\(\alpha\).]
that IL-1β excites PVN neurons indirectly via disinhibition of surrounding inhibitory GABAergic neurons.52

These conditions are altered in heart failure and hypertension. TNF-α and IL-1β are upregulated in the PVN by mechanisms that are not yet fully understood, and pro-inflammatory cytokines are known to upregulate their own receptors.53 We know that IL-6 receptors are upregulated in the PVN in heart failure rats,84 and preliminary data (unpublished) from our laboratory suggest that message for TNF-α and IL-1β receptors is also increased in the PVN in heart failure. Whether the actions of circulating cytokines in the SFO facilitate the expression of TNF-α and IL-1β receptors downstream in the PVN or whether other mechanisms are involved remains to be determined.

Limitations of the Study

We cannot exclude the possibility that an increase in circulating Ang II, precipitated by peripheral cytokine effects or cytokine-induced increases in RSNA, may have contributed to these responses; plasma Ang II levels were not measured. The fact that the lower ICA doses of TNF-α and IL-1β elicited a response at least as prominent as the higher IV doses, but only a minimal response when administered peripherally, argues against direct cytokine stimulation of RAS activity in peripheral tissues as a factor contributing to the initial rise in pressure. However, the cytokine-induced increase in RSNA, by increasing renal renin release, may have resulted in increased levels of circulating Ang II that may have contributed to the late sustained responses. In addition, we cannot exclude the possibility that leakage of the peripherally injected TNF-α and IL-1β through the injured BBB allowed access to other central nuclei that may have contributed to the residual responses we observed in the SFO-lesioned rats. We did not investigate the expression of cytokine receptors in other hypothalamic nuclei. Finally, although the SFO lesion substantially reduced the cardiovascular and sympathetic responses to acutely administered TNF-α and IL-1β, we cannot exclude a role for other CVOs and other mechanisms (induction of COX-2/PGE2 in the microvasculature; passage of cytokines across the BBB) in the residual responses. And importantly, in chronic inflammatory disease states with persistently elevated plasma cytokine levels, other central and peripheral mechanisms may assume a more prominent role.

Perspectives

This study identifies the SFO as an essential central nervous system site at which systemically administered proinflammatory cytokines act to influence cardiovascular function and sympathetic activity in normal rats. We propose that the SFO may serve a similar function in heart failure, in which circulating cytokines are chronically elevated. The SFO is a likely site for central interactions between circulating cytokines and Ang II, as well as locally produced brain cytokines and the brain RAS, that may determine the activity of the presym pathetic and neuroendocrine neurons downstream in the PVN. Other mechanisms by which cytokines might act within the SFO to increase sympathetic discharge are induction of COX-2 and synthesis of PGE254 by endothelial cells, microglia, or perivascular macrophages, and induction of inflammatory chemokines25 whose receptors are abundant in the SFO and which have recently been shown to contribute to sympathetic drive in heart failure.86 Further investigations are needed to determine the molecular and cellular mechanisms underlying the effects of blood-borne cytokines on the SFO and downstream autonomic nuclei, and the role of the SFO in inflammatory cardiovascular disease states.

Sources of Funding

This work was supported in part by the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research and Development. This work was also supported by the National Heart, Lung and Blood Institute and the National Institute of Mental Health of the National Institutes of Health under award numbers R01HL073986 (to R.B.F.), R01HL096671 (to R.B.F.), PO1HL014388 (to A.K.J.), R01HL098207 (to A.K.J.) and R01MH080241 (to A.K.J.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Disclosures

None.

References

Wei et al  SFO and Blood-Borne Cytokines 125

Novelty and Significance

What Is New?

• Intracarotid artery and IV injections of tumor necrosis factor-α or interleukin-1β induce pronounced increases in BP, HR, and RSNA that are largely dependent on an intact subfornical organ.

• Receptors for tumor necrosis factor-α and interleukin-1β are abundantly expressed in neurons and astrocytes in the subfornical organ.

What Is Relevant?

• Systemic inflammation contributes to activation of the sympathetic nervous system in hypertension and heart failure.

• This study demonstrates a major role for the subfornical organ as an interface between systemic inflammation and sympathetic activation.

Summary

The data suggest that the subfornical organ senses circulating cytokines and regulates sympathetic and hemodynamic responses to peripheral inflammation in cardiovascular disease states.


22. Banks WA, Ortiz L, Plotkin SR, Kastin AJ. Human interleukin (IL) 1 alpha, murine IL-1 alpha and murine IL-1 beta are transported from blood to brain in the mouse by a shared saturable mechanism. J Pharmacol Exp Ther. 1991;259:988–996.


Subfornical Organ Mediates Sympathetic and Hemodynamic Responses to Blood-Borne Proinflammatory Cytokines

Shun-Guang Wei, Zhi-Hua Zhang, Terry G. Beltz, Yang Yu, Alan Kim Johnson and Robert B. Felder

_Hypertension_. 2013;62:118-125; originally published online May 13, 2013;
doi: 10.1161/HYPERTENSIONAHA.113.01404

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/62/1/118

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2013/05/13/HYPERTENSIONAHA.113.01404.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/
SUPPLEMENT

to

Subfornical Organ Mediates Sympathetic and Hemodynamic Responses to Blood-borne Pro-Inflammatory Cytokines

Shun-Guang Wei,1 Zhi-Hua Zhang1, Terry G. Beltz,2 Yang Yu1, Alan Kim Johnson2

and Robert B. Felder1,3

Department of Internal Medicine, University of Iowa Carver College of Medicine1

Department of Psychology, University of Iowa2

and

Veterans Affairs Medical Center3

Iowa City, IA 52242

Running Title: SFO and blood-borne cytokines
MATERIALS AND METHODS

Surgical preparations

Preparation for SFO Lesion: SFO lesions (SFO-x) were performed using methods previously described.1 Rats were anesthetized with ketamine plus xylazine (90 mg/kg+10 mg/kg, IP). The animals were secured in a stereotaxic apparatus (Kopf Instruments; Tujunga, CA). The skull was exposed by a midline incision and leveled between the bregma and lambda. A 3.0-mm trephine hole was made at bregma. Lesions of the SFO were made by means of three penetrations along the midline using the following stereotaxic coordinates in mm, with reference to bregma and the ventral surface of midsagittal sinus: -0.4, -4.3; -0.7, -4.5; -1.0, -4.7. A monopolar electrode made of tungsten wire (0.008” in diameter, A-M Systems, Sequim, WA), insulated except at the tip, was lowered to the points described and anodal current was passed through the bare tip at 1 mA for 8 s per penetration. The reference electrode was placed in the rectum. In sham-operated SFO lesion (SFO-s) surgery, the electrode was placed 1.0 mm above the target coordinates and no current was passed. Experimentation began 1–3 wk later.

Sterile techniques were used to perform all surgical procedures, and animals were treated for postoperative pain with subcutaneous injection of buprenorphine (0.03 mg/kg, Q 12 h ×2, then as needed).

Preparation for Hemodynamic and Sympathetic Recording: Preparations for electrophysiological recording in rats have been reported previously.2 In brief, after rats were anesthetized with urethane (1.5 g/kg, IP, supplemented as needed with 0.1g/kg), a catheter was implanted into left femoral artery to measure blood pressure (BP) and derived heart rate (HR). Via a left flank incision, a renal nerve was exposed, dissected free from surrounding tissue, and placed on bipolar silver wire recording electrodes to record renal sympathetic nerve activity (RSNA). Nerve and electrodes were held together with Kwik-Cast silicon sealant (WPI, INC, Sarasota, FL) applied before the incisions were closed. A heating pad and heat lamp were used to maintain body temperature at 37 ± 1°C.

Histology

At the conclusion of the experiments, the animals that underwent the SFO lesion or sham lesion were deeply anesthetized with overdose of urethane and decapitated. The brain was rapidly removed and fixed with 4% paraformaldehyde for 48 hours, and then transferred to 30 % sucrose solution for another 48 hours. Frozen serial sagittal sections (30 µm) through the SFO region were obtained with a cryostat. The SFO lesion was confirmed by a light microscopy.

Brain tissue preparation

To obtain tissues for immunofluorescent staining, rats were anesthetized with urethane (1.5 g/kg, IP) and transcardially perfused with 4% paraformaldehyde. The brain was removed and post-fixed with 4% paraformaldehyde for another 12 hrs, and then transferred to 30 % sucrose solution for another 48 hrs. After that, brains were embedded with OCT and rapidly frozen in alcohol chilled dry ice. Coronal sections (16 µm) of target brain tissues were made with a cryostat and stored at −80 °C.
**Immunofluorescence**

Immunofluorescent staining was used to examine the expression of the p55 TNF-α receptor (TNFR1) and IL-1 receptor accessory protein (IL-1RAcP), a marker of the IL-1 receptor (IL-1R), in the subfornical organ (SFO) and hypothalamic paraventricular nucleus (PVN) in normal rats. The sections were incubated with the primary antibodies, the rabbit polyclonal antibodies to TNFR1 (ab19139, 1: 200, Abcam), IL-1RAcP (I8153, 1: 200, Sigma-Aldrich), and/or the mouse monoclonal antibodies to astrocyte marker GFAP (#3670, Cell Signaling) and neuronal marker NeuN (MAB377,1:300, Millipore) followed by secondary antibodies Alex Fluor 488 goat anti-rabbit IgG  (A-11070, 1:200, Invitrogen) and Alex Fluor 568 goat anti-mouse IgG (A-11003, 1:200, Invitrogen). Immunofluorescent staining was visualized with a confocal laser-scanning microscope (Zeiss LSM 710, Carl Zeiss, Inc).

**Data acquisition**

A Cambridge Electronics Design laboratory interface (CED, model 1401; Cambridge, UK) connected to a personal computer was used to acquire the data. The raw RSNA was amplified by Grass preamplifier (Grass Instrument Co, Quincy, MA) and then was rectified and integrated by a Paynter filter (20-ms time constant, BAK Electronics; Germantown, MD) before being passed to the CED 1401 device. BP signals were transferred to CED 1401 via a pressure transducer and a Gould TA240S chart recorder (Gould Instruments, Valley. View, OH). HR was derived from the frequency of the arterial pressure pulses. Digitized data were stored for subsequent off-line analysis with Spike2 software (CED).

The electrophysiological and hemodynamic data were analyzed with CED Spike2 software. Mean blood pressure (MBP, mmHg), HR (bpm), and integrated RSNA (mV) were averaged over 5 min intervals after intracarotid or intravenous drug administration and compared with baseline values averaged over 5 min intervals immediately preceding each intervention. Changes in integrated RSNA are reported as a percentage of baseline control.

**Statistical Analysis**

All values are expressed as the means ± SEM. The significance of differences among groups was analyzed by 2-way repeated-measure ANOVA followed by post hoc Fisher’s test. Student’s t-test was used to determine statistical significance between paired data for a single comparison. p< 0.05 was considered to indicate statistical significance.

**REFERENCES**

Figure S1. Representative tracings showing that intracarotid artery (ICA) injection of vehicle did not change BP, HR and RSNA in SFO-s (A) and SFO-x (B) rats. BP: blood pressure; HR: heart rate; RSNA: renal sympathetic nerve activity, windowed (spikes/s) and integrated (mV). SFO-s: sham-operated SFO-lesioned rat; SFO-x: SFO-lesioned rat. Scale bar: 5 min.
Figure S2. Typical histological graphic shows the electrolytic lesion on the SFO. A: SFO sham lesion; B: SFO lesion.
Figure S3. A, a histological image showing a missed SFO lesion. B, a representative electrophysiological recording tracing showing that the excitatory responses in BP, HR and RSNA in response to ICA injection of TNF-α remain in the missed lesion rat. BP, blood pressure; HR, heart rate; RSNA, renal sympathetic nerve activity; ICA, intracarotid artery. TNF-α, tumor necrosis factor-alpha. Scale bar: 20 min.
Figure S4. The representative confocal images from hypothalamic paraventricular nucleus (PVN) showing the expression of p55 TNF-α receptor (TNFR1, A) and IL-1 receptor accessory protein (IL-1RAcP), a subunit of the IL-1 receptor (B) in the normal rats. There was no significant expression of TNFR1 in the PVN. IL-1R was sparsely expressed only in the magnocellular subdivision of the PVN. Scale bar: 200µm. 3rd ventricle to left.
Figure S5. The representative negative confocal control images from subfornical organ (SFO) showing: A, Second antibodies to Alexa-488 and 568 only; B, Primary antibody to neuronal marker NeuN plus secondary antibodies to Alexa-488 and 568; C: Primary antibody to astrocyte marker GFAP plus secondary antibodies to Alexa-488 and 568. Scale bar: 200µm.