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Hypothalamic CRF and Norepinephrine Mediate Sympathetic and Cardiovascular Responses to Acute Intracarotid Injection of TNF- α in the Rat

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Abstract

Systemic administration of tumour necrosis factor - α (TNF- α) induces the release of norepinephrine (NE) in the paraventricular nucleus (PVN) of hypothalamus and an increase in expression of corticotrophin-releasing-factor (CRF) and CRF type 1 receptors. We explored the hypothesis that CRF and NE in PVN mediate the cardiovascular and sympathetic responses to acute systemic administration of TNF- α . In anaesthetised rats, the increases in arterial pressure and heart rate induced by intracarotid artery injection of TNF- α were attenuated by intracerebroventricular (ICV) injection of either the α 1-adrenergic antagonist prazosin or the CRF antagonist α -helical CRF. Prazosin blocked the TNF- α -induced increase in renal sympathetic nerve activity (RSNA), while α -helical CRF substantially reduced the RSNA response. Conversely, CRF and the α 1-adrenergic agonist phenylephrine (PE), administered ICV, both elicited increases in PVN neuronal activity, RSNA, arterial pressure and heart rate. Microinjection of CRF and PE directly into PVN evoked smaller responses. These results are consistent with the hypothesis that NE and CRF in the PVN mediate the cardiovascular and sympathetic responses to acute systemic administration of TNF- α .

Keywords

corticotrophin releasing factor; paraventricular nucleus of hypothalamus; tumour necrosis factor - α (TNF- α)

Introduction

In a seminal report (1), Brown and Fisher summarised their experiments demonstrating that centrally administered corticotrophin releasing factor (CRF) elicits cardiovascular and autonomic responses, in addition to the release of adrenocorticotrophic hormone (ACTH) and corticosterone. The generalised state of neuroendocrine and cardiovascular excitation induced by intracerebroventricular injection of CRF led these investigators to postulate that this “single peptide” might coordinate the response to a variety of stressors (1). Brown and Fisher and colleagues further determined that the cardiovascular effects of centrally administered CRF were not dependent upon activation of the pituitary adrenal axis, but rather upon sympathetic and vagal efferent mechanisms (2). CRF also increased plasma renin activity, likely via a sympathetic efferent mechanism, but not plasma vasopressin.

More recent studies have demonstrated that CRF neurones in the paraventricular nucleus of the hypothalamus (PVN) are a primary target for circulating pro-inflammatory cytokines (3-6). The central mechanism by which acute systemic injections of pro-inflammatory cytokines elicit a glucocorticoid response is now well delineated. TNF- α and interleukin-1 beta (IL-1 β) act upon perivascular cells of the cerebral circulation (7) to induce synthesis of prostaglandin E2 (PGE₂). PGE₂ crosses the blood-brain barrier (8) to activate a select group of noradrenergic neurones in rostral ventrolateral medulla (RVLM) that project to the hypothalamus (9), where release of norepinephrine (NE) from their axon terminals stimulates PVN neurones to express CRF (10-13). These neuroendocrine CRF neurones project to median eminence to effect ACTH release from the anterior pituitary, ultimately regulating glucocorticoid release from the adrenal gland.

In contrast, the mechanisms by which the pro-inflammatory cytokines activate the sympathetic nervous system are still poorly understood. Most of the CRF neurones in PVN are committed to a neuroendocrine function (14), but others project directly to RVLM (15) and to the intermediolateral cell column (IML) of the spinal cord (16) - central nuclei containing presympathetic neurones (17). Unlike the neuroendocrine CRF neurones, these long descending preautonomic CRF neurones are not subject to feedback regulation by glucocorticoids (16). Thus, while preautonomic and neuroendocrine CRF neurones co-mingle in the PVN, they may be regulated differently.

The CRF type 1 receptors (CRF-R1) that mediate autonomic responses to CRF have only a scant presence in the PVN under normal conditions (18). Under conditions of stress, or even in the presence of increased CRF, CRF-R1 in the PVN are dramatically upregulated (19-21). Thus, in response to acute systemic administration of TNF- α or IL-1 β , it seems likely that CRF might act upon CRF-R1 receptors in PVN to increase sympathetic drive and that NE, released into PVN by noradrenergic neurones ascending from the brain stem, might facilitate that response. The link between physical and psychosocial stressors, pro-inflammatory cytokines and NE in critical regions of the central nervous system is well established (22,23).

In a previous study (24), we found that acute intracarotid injection of TNF- α elicits cardiovascular and renal sympathetic excitation in anaesthetised rats. In the present study, we investigated the role of CRF and NE as putative mediators of this autonomic response.

Materials and methods

Experiments were performed on adult male Sprague-Dawley rats (300-350 g). The animals were housed in the University of Iowa Animal Care Facility and exposed to a normal 12:12-h light-dark cycle. The studies were performed in accordance with the "Guiding Principles for Research Involving Animals and Human Beings" of the American Physiological Society (25). The experimental procedures were approved by the University of Iowa Institutional Animal Care and Use Committee.

General surgical preparation

Rats were anaesthetised with urethane (1.5 g/kg, ip), and supplemental doses of urethane (0.1-0.3 g/kg, ip or iv) were given when increases in arterial pressure (AP), heart rate (HR), or respiratory rate were observed during surgery or recording. The level of anaesthesia was periodically reassessed during the surgical procedures and experimental recording by testing for nociceptive reflex responses and by continuously monitoring AP and HR. The left femoral artery was cannulated with PE-50 tubing filled with heparinized saline (20 U/ml) and connected to a pressure transducer for the recording of AP with a Hewlett-Packard 7754A chart recorder (HP Medical Products Group, Andover, MA). The left femoral vein

was cannulated with PE-20 tubing for the administration of drugs. A rostrally directed PE-20 cannula was inserted into the left common carotid artery, with the tip placed in the bifurcation for intracarotid artery (ICA) injection as previously reported (24,26). Animals were intubated and breathed spontaneously. Core temperature was maintained at $37 \pm 0.3^\circ\text{C}$ with a rectal thermometer and a temperature controller (model K-100, Baxter Healthcare, Valencia, CA). The head was fixed in a stereotaxic frame (David Kopf Instrument, Tujunga, CA). Stereotaxic coordinates for 3rd ventricular injections and recording and microinjection sites in PVN and RVLM were derived from the rat atlas of Paxinos and Watson (27).

Intracerebroventricular (ICV) injections

A 23-gauge stainless steel guide cannula was lowered into the 3rd cerebral ventricle using standard stereotaxic procedures. The coordinates with respect to bregma were -1.8 mm antero-posterior, 1.5 mm lateral from the midline, and -9.0 mm dorsoventral from the dura, with the cannula angled 10° towards midline. ICV injections were made in a volume of 2 μl over 10 seconds using a 29-gauge tubing. The ICV position of the cannula was confirmed by the staining of all four ventricles after injection of 2 μl pontamine sky blue at the end of the experiments.

PVN and RVLM microinjections

In two experimental groups, a 29-gauge guide cannula was inserted bilaterally 0.5 mm above the PVN or RVLM region. A 35-gauge (128 μm OD; 51.2 μm ID) stainless steel injection cannula was attached to PE-10 tubing, which was then connected to a 0.5- μl Hamilton microsyringe. The tip of the injection cannula was inserted into the guide cannula and then adjusted to a length extending 0.5 mm beyond the tip of the guide cannula. Bilateral microinjections were made in a volume of 100 nl over 10 sec. The PVN injection cannulae were positioned 1.8 mm posterior to bregma, with one cannula 0.4 mm from midline and parallel to midline and the other 1.9 mm from midline and angled 10° toward midline, 7.6 mm ventral to dura. The RVLM injection cannulae were positioned 12.0 mm posterior to bregma, 1.9 mm from midline, and 9.0 mm ventral to dura. At the end of the experiments, 100 nl 2% pontamine sky blue was injected into the same location for histological verification of injection sites.

Electrophysiological recording procedures

A small craniotomy was made above the region of PVN, and a glass micropipette was placed in the left PVN to record extracellular single-unit activity, using previously described techniques (24,26). Stereotaxic coordinates for PVN recording were 1.6-2.1 mm posterior to bregma, 0.3-0.5 mm from midline, and 7.0-8.0 mm ventral to dura. The last unit recorded in each experiment was marked with iontophoresis of pontamine sky blue for subsequent determination of recording sites.

Recordings of RSNA were obtained from the left renal nerve using methods previously described (24,28). In brief, the left kidney was exposed through a flank incision. A branch of the renal nerve was dissected free from surrounding tissue and placed on bipolar silver wire recording electrodes. When an optimal signal-to-noise ratio was established, the electrode and the renal nerve were covered with silicon sealant (World Precision Instruments, Sarasota, FL). The electrodes were sutured to the back muscles. Renal nerve activity was amplified (model P511, Grass Instrument; Quincy, MA) and displayed on an oscilloscope (TDS 3014, Tektronix, Beaverton, OR). The noise level was finally determined at the end of experiment after injection of hexamethonium (30 mg/kg iv). The net value of RSNA was calculated by subtracting the background noise from the actual recorded value during the experiment. The recording session began at least an hour after completion of the surgical preparation.

Drugs

Hexamethonium bromide, phenylephrine hydrochloride (PE) and prazosin were purchased from Sigma (St. Louis, MO). Recombinant rat TNF- α was obtained from Research Diagnostic Inc. (Flanders, NJ). CRF and α -helical CRF (9-41) were purchased from Phoenix Pharmaceuticals Inc. (Belmont, CA). The drugs were dissolved in artificial cerebrospinal fluid (aCSF) for ICV injection or for direct microinjection into PVN or RVLM and in saline for ICA and intravenous injections. The same volume of normal saline or aCSF was administered as a control for the ICA or ICV injections and tissue microinjections. The doses of drugs used in the present study were derived from previous studies from our laboratory and others and were optimised in preliminary experiments.

Experimental protocols

Protocol 1—In 21 rats, we tested the MAP, HR and RSNA responses to ICA TNF- α (0.5 μ g/kg) before and 5 minutes after 3rd ventricular administration of the CRF antagonist α -helical CRF (20 μ g/kg in 2 μ l, ICV, n=7) or the α 1-adrenergic antagonist prazosin (1 μ g, n=9), or Veh (2 μ l, n=13).

Protocol 2—In 10 rats, we tested the effects of microinjecting CRF (2 μ g in 2 μ l, ICV; n=6), PE (1 μ g, ICV; n=6) or artificial cerebrospinal fluid (Veh, 2 μ l, n=5) into the 3rd ventricle on PVN neuronal activity, MAP, HR and RSNA.

Protocol 3—In 17 rats, we tested the effects of microinjecting CRF (0.2 μ g in 100 nl, n=6), PE (0.2 μ g, n=9) or Veh (100 nl, n=5) directly into bilateral PVN on MAP, HR and RSNA.

Protocol 4—In 7 rats, we tested the effects of microinjecting CRF (0.2 μ g in 100 nl, n=7) and Veh (100 nl, n=5) directly into bilateral RVLM on MAP, HR and RSNA.

To minimise the number of animals used, most rats underwent 1-3 interventions, usually vehicle first followed by treatment(s), with full recovery of recorded variables between treatments. In some experiments, more than one PVN unit was recorded and tested. *N* values in the figures and legends refer to number of treatments or interventions rather than number of rats tested.

Anatomy/histology

At the conclusion of each experiment, the rat was killed with an overdose of urethane. The brain was removed and fixed in a 10% formalin solution for at least 3 days and then sectioned (40 μ m) on a cryostatic microtome (OM2563, Triangle Biomedical Sciences, Durham, NC). The sections were thaw-mounted on microscope slides and then stained with 1% aqueous neutral red. The recording and microinjection sites marked with pontamine sky blue were identified with a light microscope, and the locations of other recording sites were extrapolated with respect to this reference point. Recording and injection sites were plotted on representative schematic tracings of the PVN and RVLM, based on the rat atlas of Paxinos and Watson (27).

Data acquisition and analysis

The arterial pressure signal, the rectified and integrated voltage from the renal nerve recording, and the transistor-transistor logic pulses indicating PVN single unit activity were fed into an on-line data-acquisition system consisting of a Cambridge Electronics Design (CED, Cambridge, UK) 1401 Plus computer interface coupled with a Gateway Pentium personal computer. Mean arterial pressure (MAP) and HR were derived from the arterial pressure tracing.

MAP, HR, RSNA, and single-unit discharge were averaged over 1-min intervals. A 3-min baseline was used as control. Because of variations from animal to animal, the MAP and HR data are presented as changes from baseline instead of absolute values. Changes in RSNA and PVN neuronal activity were calculated as a percent change from the baseline activity. The statistical significance of differences from baseline and differences between groups were determined by one-way or two-way repeated-measures ANOVA followed by post hoc Tukey test. Values are expressed as means $\pm$ SE. $P < 0.05$ was considered to indicate statistical significance.

Results

ICV administration of α -helical CRF and prazosin: Effects on cardiovascular and sympathetic responses to intracarotid artery injections of TNF- α

ICA injection of TNF- α (0.5 μ g/kg) significantly increased RSNA, HR and AP (Fig. 1A and 1C). The onset latency of this response was approximately 15 minutes with duration exceeding 90 minutes, the duration of the recording session. The responses peaked within 20-35 minutes after TNF- α administration. Grouped data (Fig. 1C) show that RSNA increased by $45.5 \pm 6.5\%$ from baseline ($n=13$, $p<0.01$) in response to TNF- α . MAP increased 20.8 ± 2.8 mmHg ($p<0.01$) from a baseline of 104.2 ± 4.9 mmHg, while HR increased 41.5 ± 5.1 bpm from baseline of 351.8 ± 10.9 bpm ($p<0.01$). Administration of the same volume of saline (5 μ l, ICA) alone had no effects on RSNA, AP or HR ($n=5$; data not shown).

After pre-treatment with the CRF antagonist α -helical CRF (20 μ g, ICV), ICA TNF- α elicited smaller increases in ($p<0.05$, vs Veh +TNF- α) MAP (11.0 ± 4.5 mmHg), HR (20.5 ± 7.8 bpm) and RSNA ($23.6 \pm 4.3\%$) (Fig 1B,C). The same dose of α -helical CRF alone did not change baseline MAP (-0.7 ± 1.7 mmHg), HR (2.4 ± 5.3 bpm) or RSNA ($-1.9 \pm 3.5\%$) (Fig. 1 C).

After pre-treatment with α 1-adrenergic antagonist prazosin (1 μ g, ICV), ICA TNF- α elicited a small ($p<0.05$) increase in HR (17.7 ± 4.6 bpm) but no change in RSNA (Fig. 2 B and C). ICV prazosin decreased MAP slightly but significantly (-7.7 ± 2.1 mmHg, $p<0.05$ vs baseline) but did not significantly alter HR (-1.4 ± 1.8 bpm) or RSNA ($0.5 \pm 3.9\%$). After the pre-treatment with ICV prazosin, ICA TNF- α induced a minimal but statistically significant increase in MAP (6.4 ± 3.5 mmHg $p<0.05$ vs baseline). The increases in MAP and HR were significantly smaller ($p<0.05$) than Veh+TNF- α .

ICV administration of CRF and PE: Effects on PVN neuronal activity, RSNA, arterial pressure and heart rate

ICV CRF (2 μ g, ICV; $n=6$) dramatically increased PVN neuronal activity ($164.2 \pm 34.1\%$ from baseline of 2.2 ± 0.2 spikes/s, $p<0.01$), and elicited increases in RSNA ($44.4 \pm 7.6\%$, $p<0.01$ vs baseline) and HR (52.2 ± 12.0 bpm from baseline of 297.8 ± 10.1 bpm, $p<0.01$) that were comparable to ICA TNF- α (Fig. 3). MAP increased (13.2 ± 2.5 mmHg from baseline of 94.6 ± 2.9 mmHg, $p<0.05$) only modestly. The responses to began within 5 min after 3rd ventricular injection and lasted more than 90 min. These responses were blocked ($n=5$) by pre-administration of the CRF receptor antagonist α -helical CRF (20 μ g, ICV) (Fig. 3 B and C), the same dose that incompletely blocked the responses to ICA TNF- α (Fig 1).

ICV administration of the α 1-adrenergic agonist phenylephrine (PE, 1 μ g, $n=6$) increased ($p<0.05$) PVN neuronal activity ($189.7 \pm 48.6\%$ from baseline of 2.3 ± 0.4 spikes/s), RSNA ($17.7 \pm 5.9\%$), MAP (8.1 ± 2.9 mmHg from baseline of 89.7 ± 2.1 mmHg,) and HR (25.8 ± 5.4 bpm from baseline of 317.6 ± 11.1 bpm) (Fig. 4). The pressor response was preceded by

a small and statistically insignificant transient drop in HR and MAP. The latency to a significant increase in the measured variables was 10-20 min with peak responses occurring about 15-45 min after injection and recovery of all variables except PVN neuronal discharge within 60 min.

Microinjection of CRF and PE in PVN: Effects on RSNA, arterial pressure and heart rate

Microinjection of CRF (0.2 μ g) directly into bilateral PVN evoked small increases ($p < 0.05$ vs baseline or Veh) in RSNA ($13.5 \pm 2.7\%$, $n=6$) and HR (27.9 ± 5.6 bpm from baseline of 314.1 ± 7.2 bpm) and no significant increase in MAP (4.7 ± 1.2 mmHg from baseline of 94.5 ± 4.7 mmHg) (Fig. 5). The increases in MAP and HR were significant 5 min after injection and lasted more than 90 min. Five control injections in brain tissue surrounding PVN had no significant effects. The same volume of Veh (100 nl) microinjected into PVN ($n=5$) induced no significant responses (Fig 5B).

Bilateral microinjection of PE (0.2 μ g) directly into PVN increased ($p < 0.05$, versus baseline) RSNA ($19.7 \pm 3.9\%$, $n=9$) and HR (18.1 ± 2.7 bpm from baseline 336.6 ± 10.7 bpm), but did not affect MAP (Fig. 6). The increases in HR and RSNA were significant within 10 minutes, peaked at 25 minutes and lasted for about 45 min. Four control injections in adjacent brain tissue dorsal and lateral to PVN had little effect. The same volume of Veh (100 nl) microinjected into PVN ($n=5$) induced no significant change in the variables measured.

Microinjection of CRF in RVLM: Effects on RSNA, arterial pressure and heart rate

We also tested the effect of CRF on sympathetic outflow from the RVLM, another important brainstem locus of pre-sympathetic neurones (17) that receives CRF-containing fibres from PVN (15). Microinjection of CRF bilaterally into RVLM (Fig. 7) elicited a small increase in MAP (7.0 ± 1.8 mmHg from baseline 94.8 ± 4.4 bpm, $p < 0.05$) and modest increases in RSNA ($15.0 \pm 6.3\%$, $p < 0.05$, $n=7$) and HR (18.0 ± 7.6 bpm from baseline of 305.7 ± 8.7 bpm), again substantially smaller than the responses evoked by ICA TNF- α (Fig 1) or by 3rd ventricular CRF (Fig 3). Four control injections in brain tissue surrounding RVLM had no significant effects on these variables. The same volume of Veh (100 nl) microinjected into RVLM ($n=5$) induced no significant responses.

Recording and microinjection sites in PVN and RVLM regions

The locations of the single unit recording sites in PVN and the microinjection sites in PVN and RVLM areas are shown in Fig. 8. There was no obvious anatomical cluster of neurones responsive to administered drugs. Microinjection sites from which responses were elicited were localised within the visual boundaries of PVN or RVLM. There was no correlation between the responses and the placement of the cannula in the sub-regions of the PVN or RVLM.

Discussion

The principal new finding of this study is that the increase in sympathetic nerve activity elicited by intra-carotid administration of TNF- α is mediated at least in part by NE and CRF, which also mediate the endocrine responses to pro-inflammatory cytokines. TNF- α -induced increases in HR, MAP and RSNA were abrogated to a similar extent by ICV administration of prazosin and α -helical CRF, though the RSNA response appeared to be more sensitive to prazosin. These observations suggest that a parsimonious central mechanism regulates both the endocrine and the autonomic responses to acute systemic administration of TNF- α .

Since CRF and noradrenergic neurones and their receptors are widely distributed in the brain, ICV administration of prazosin and α -helical CRF does not permit localisation of their effects to a specific nuclear region. However, since the endocrine (i.e., glucocorticoid) response to an acute cytokine challenge ultimately depends upon noradrenergic activation of CRF-containing neurones in the PVN, we tested the ability of CRF and NE to excite PVN neurones and to elicit a sympatho-excitatory response. ICV CRF and NE elicited significant increases in PVN neuronal activity, RSNA, MAP and HR. However, when injected directly into the PVN, both agonists increased RSNA and HR, but neither had any effect on MAP. These results are consistent with the view that CRF and NE actions in the PVN contribute to the autonomic responses to acute systemic TNF- α , but that other sites and perhaps other mechanisms may be involved in the arterial pressure response.

One likely alternative site of action is the RVLM, which receives CRF projections from PVN (15) and is an important hindbrain site regulating sympathetic outflow (29,30). A previous microinjection study (15) demonstrated that CRF elicits a pressor response when injected into this nucleus. We observed a similar effect. Like the PVN injections, bilateral microinjections of CRF into RVLM increased RSNA and HR. However, the RVLM injections also increased MAP. Thus, it is conceivable that NE activates CRF-producing neurones in PVN that project to and stimulate presympathetic neurones in the RVLM. NE was not microinjected into RVLM, since there is no intrinsic neuronal source of CRF.

In considering these results, it is important to realise that the differences in magnitude and duration of responses to CRF, NE and their respective antagonists may reflect a number of experimental variables, including dose, route of administration, and alternative sites and mechanisms of action. For example, we do not know what dose of microinjected NE would be required to elicit tissue CRF concentrations in PVN comparable to those induced by intracarotid TNF- α or local microinjection of CRF. Similarly, the different distributions and functions of CRF and NE fibres within the brain (10-13) make it unlikely that one would observe identical responses to the CRF and NE antagonist, even though this study clearly demonstrates that both systems are involved in the regulation of sympathetic drive. Thus, ICV prazosin blocked the RSNA response to TNF- α , but ICV α -helical CRF failed to do so even though the dose of α -helical CRF was sufficient to block the effects of ICV CRF. This may reflect a higher tissue concentration of CRF or of CRF-R1 induced by intracarotid TNF- α than by ICV CRF. It is tempting to speculate that α -1 adrenergic receptors have a predominant influence over the renal sympathetic response to TNF- α , but experimental uncertainties limit the validity of that conclusion.

The incomplete blockade of the TNF- α effect by α -helical CRF and prazosin also leaves room to consider alternative mechanisms by which cytokines might activate sympathetic drive. One such mechanism might be direct activation of hypothalamic neurones by the prostaglandin E₂ (PGE₂) produced in perivascular macrophages of the blood brain barrier (31), in response to cytokine induction of cyclooxygenase-2 (COX-2) enzyme activity. Receptors for PGE₂ are present in the hypothalamus (32,33) and ICV administration of PGE₂ induces *c-fos* expression in the PVN (34) and a sympatho-excitatory response (24,35). PGE₂ may act by inhibiting GABAergic neurones that normally restrain PVN neuronal activity (36). In a previous study (24), we found that ICV administration of a cyclooxygenase inhibitor prevents the activation of PVN neurones and the increases in HR, AP and RSNA elicited by ICA TNF- α . Thus, cytokine induction of perivascular macrophage COX-2 activity and PGE₂ production is another potential mechanism for TNF- α activation of the sympathetic nervous system. PGE₂ may also induce CRF and CRF-R1 activity (34), but whether the sympatho-excitatory effects of PGE₂ are linked specifically to upregulation of CRF and its receptors remains to be determined.

Perspectives

The realisation that inflammatory mediators play a major role in the pathophysiology of cardiovascular diseases (37–41) provides new impetus for the study of cytokine-induced autonomic responses. The cardiovascular, autonomic and metabolic consequences of chronic activation of CRF neurones may have a devastating impact on the clinical course of cardiovascular diseases. Pro-inflammatory cytokines are increased in myocardial infarction (42) and heart failure (43), and in associated conditions such as hypertension (40), obesity (44), obstructive sleep apnea (45), diabetes mellitus (46) and depression (47). Shifts in the balance of autonomic activity toward sympathetic excitation are directly linked to morbidity and mortality in myocardial infarction and congestive heart failure (48,49). In heart failure, for example, augmented sympathetic drive is associated with depression of myocardial contractility (50), myocyte loss (51), increased peripheral resistance, and increased risk of sudden cardiac death (48,49). The present study suggests that TNF- α contributes to sympathetic excitation by increasing NE and CRF activity in the PVN. The possibility that chronic activation of hypothalamic mechanisms by TNF- α and other pro-inflammatory cytokines might promote the pathophysiology of cardiovascular diseases is underappreciated and demands further investigation.

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Fig. 1. Involvement of central CRF receptors in TNF- α -induced sympathetic and cardiovascular responses

Representative tracings showing: A, the TNF- α -induced increases in renal sympathetic nerve activity (RSNA), arterial pressure (AP) and heart rate (HR); B, Prior ICV administration of CRF antagonist α -helical CRF significantly reduces the ICA TNF- α induced cardiovascular and sympatho-excitatory responses. C: grouped data showing that ICV α -helical CRF (n=7) partially blocked responses to ICA TNF- α (n=13). ICV α -helical CRF alone (n=5) did not change baseline level of MAP, HR and RSNA. Data are shown as in changes from baseline level. The arrows indicate the ICA injection. * p < 0.05, vs baseline; † p < 0.05, ICV α -helical CRF + ICA TNF- α vs ICV Veh + ICA TNF- α .

Fig. 2. Involvement of central α 1-adrenergic receptors in TNF- α -induced sympathetic and cardiovascular responses

Representative tracings showing: A, TNF- α -induced increases in RSNA, AP and HR; B, reduction of these responses by prior ICV administration of α 1-adrenergic antagonist prazosin. C: grouped data showing that ICV prazosin (n=9) blocked RSNA response induced by ICA TNF- α , and partially blocked cardiovascular responses to ICA TNF- α . ICV prazosin alone (n=5) had no effect on HR or RSNA, but AP was mildly reduced. Data are shown as in changes from baseline level. The arrows indicate the ICA injection. * p < 0.05, vs baseline; † p < 0.05, ICV prazosin + ICA TNF- α vs ICV Veh + ICA TNF- α .

Fig. 3. Effects of ICV injection of CRF on PVN neuronal activity and sympathetic activation
 Representative tracing showing ICV administration of CRF remarkably increased PVN neuronal activity, RSNA, AP and HR (A), and these responses were blocked by ICV CRF antagonist α -helical CRF (B). C: grouped data showing ICV CRF (n=6) significantly increase PVN neuronal firing, RSNA and HR with smaller increase of MAP. ICV CRF antagonist α -helical CRF (n=5) completely abolished CRF-induced sympathoexcitatory responses. The arrows indicate the ICV injection. * $p < 0.05$, vs baseline; † $p < 0.05$, ICV α -helical CRF + ICV CRF vs ICV Veh + ICV CRF.

Fig. 4. Effects of ICV injection of α_1 -adrenergic agonist phenylephrine on PVN neuronal activity and sympathetic activation

A: Representative tracing showing ICV injection of α_1 -adrenergic agonist phenylephrine (PE) increases paraventricular nucleus (PVN) neuronal activity, RSNA, AP and HR. B: grouped data showing ICV PE (n=6) significantly increase PVN neuronal activity, RSNA, MAP and HR compared with same volume of vehicle injection (ICV Veh, n=5). The arrows indicate the ICV injection. * $p<0.05$, vs baseline; † $p<0.05$, ICV PE vs ICV Veh.

Fig. 5. Effects of direct PVN microinjection of CRF on sympathetic and cardiovascular activity
 A: Representative tracing showing the effect of bilateral microinjection of CRF directly into PVN on RSNA, HR and AP. B: grouped data showing the significant increases in RSNA and HR following CRF microinjection (n=6) into PVN compared with vehicle treatment (PVN Veh, n=5). There is only slight elevation of MAP after CRF microinjection. The arrows indicate the PVN microinjection. * $p<0.05$, vs baseline; † $p<0.05$, PVN CRF vs PVN Veh.

Fig. 6. Effects of direct PVN microinjection of α_1 -adrenergic agonist phenylephrine on sympathetic and cardiovascular activity

A: Representative tracing illustrating the effect of microinjection of phenylephrine (PE) directly into bilateral PVN on RSNA, HR and AP. B: grouped data showing the significant increases in RSNA and HR following PE microinjection (n=9) into bilateral PVN compared with vehicle treatment (PVN Veh, n=5). No significant elevation of MAP after PE microinjection. The arrows indicate the PVN microinjection. * $p < 0.05$ vs baseline; † $p < 0.05$, PVN PE vs PVN Veh.

Fig 7. Effects of direct RVLM microinjection of CRF on sympathetic and cardiovascular activity
 A: Representative tracing showing the effect of bilateral microinjection of CRF directly into RVLM on HR, AP, and RSNA. B: grouped data showing the significant increases in HR, MAP and RSNA following CRF microinjection (n=7) into RVLM compared with vehicle treatment (RVLM Veh, n=5). The arrows indicate the RVLM microinjection. * $p < 0.05$ vs baseline; † $p < 0.05$, RVLM CRF vs RVLM Veh.

Fig. 8. Schematic reconstruction of recording and microinjection sites in PVN (A) and RVLM (B) regions

Locations shown are: PVN neurones that are responsive to ICV CRF (●) and un-responsive to ICV CRF after ICV α -helical CRF (○); PVN neurones responsive to ICV PE (▲); PVN neurones unresponsive to ICV Veh (△); microinjection sites of CRF (■) and Veh (□) within PVN (bilateral) and RVLM (bilateral); microinjection sites of PE (filled diamond) within PVN (bilateral). The distance from bregma is indicated. Sections are modified from Paxinos and Watson (27). f, fornix; IO, inferior olive; AH, anterior hypothalamus, LPGi, lateral paragigantocellular nucleus; NA, nucleus ambiguus; NTS, nucleus of the solitary tract; py, pyramidal tract; 3V, 3rd ventricle.