Effect of acute adrenalectomy on sympathetic responses to peripheral lipopolysaccharide or central PGE$_2$


Department of Pathology and 2Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3

MacNeil, B. J., A. H. J. Ansen, A. H. Greenberg, and D. M. Nance. Effect of acute adrenalectomy on sympathetic responses to peripheral lipopolysaccharide or central PGE$_2$. Am J Physiol Regulatory Integrative Comp Physiol 278: R1321–R1328, 2000.—The impact of plasma corticosterone levels on the sympathetic nervous system (SNS) response to intravenous lipopolysaccharide (LPS) or intracerebroventricular injections of PG was studied in anesthetized (urethane-chloralose) male Sprague-Dawley rats. For this, electrophysiological recordings of splenic and renal nerves were completed in control or adrenalectomized (ADX) rats. LPS (10 µg iv) similarly increased splenic and renal nerve activity in control rats with a shorter onset latency for the splenic nerve. Acute ADX enhanced the response of both nerves to LPS (P < 0.005) and reduced the onset latency of the renal nerve (P < 0.05). PGE$_2$ (2 µg icv) rapidly increased the activity of both nerves but preferentially (magnitude and onset latency) stimulated the renal nerve (P < 0.05). The magnitude of the splenic nerve response to PGE$_2$ was unaffected by ADX. Unexpectedly, PGE$_2$ was less effective at stimulating renal nerve activity in ADX animals relative to intact controls (P < 0.05). Pretreatment of ADX rats with a CRF antagonist ([D-Phe$^1$, Nle$^{1-38}$, Ca-MeLeu$^{22-}$.CRF-(12–41)] reversed this effect such that the renal nerve responded to central PGE$_2$ to a greater extent than the splenic nerve (P < 0.05), as was the case in non-ADX rats. These data indicate that enhanced sensitivity of central sympathetic pathways does not account for the enhanced SNS responses to LPS in ADX rats. Also, a CRF-related process appears to diminish renal sympathetic outflow in ADX rats.

Electrophysiology; splenic; renal; nerve; corticotropin-releasing factor antagonist; corticosterone

Peripheral injection of lipopolysaccharide (LPS) stimulates the production and secretion of cytokines that can be detected in the plasma and brain (12, 26). LPS also activates the hypothalamic-pituitary-adrenal (HPA) axis, possibly mediated by the aforementioned cytokines, and central PG synthesis (32). The primary outcome of HPA axis stimulation is an increase in the circulating level of corticosterone, which in turn reduces HPA axis activity through a well-described negative-feedback loop by inhibiting secretion of corticotropin-releasing factor (CRF; see Ref. 6). CRF is generally thought to represent the final common pathway of acute HPA axis stimulation (32). Corticosterone is also a negative modulator of immune cell function, including cytokine production (22). Besedovsky and colleagues (3) have described a complete counterregulatory system in which an increase in plasma interleukin-1 activates the HPA axis and elevates plasma corticosterone levels. The increase in corticosterone will affect two convergent processes. In the central nervous system (CNS), the negative feedback from an elevation of corticosterone will counteract CRF release. Simultaneously, cytokine production will be inhibited and thereby reduce further stimulation of the HPA axis. The significance of circulating corticosterone in regulating cytokine production is underlined by the finding that removal of the adrenal glands [adrenalectomy (ADX)] leads to excessive cytokine production after LPS, a response that can be lethal unless normalized by glucocorticoid replacement (12, 26).

In addition to the HPA axis, systemic injection of LPS also activates the sympathetic nervous system (SNS; see Refs. 18 and 19). Cytokines are one potential mediator of this response given that sympathetic discharge is increased after peripheral or central injection of various cytokines (14, 24, 33). PG synthesis within the CNS is also involved, since centrally injected indomethacin blocks the sympathetic response to LPS (19). Moreover, intracerebroventricular injection of PGE$_2$ elicits an immediate increase in sympathetic nerve discharge (2, 19). Thus ADX would be expected to increase LPS-induced sympathetic activation, and a potential mechanism would be enhanced production of intermediary factors (cytokines, PGs) after removal of the inhibitory effects of glucocorticoids on their synthesis. However, there are substantial changes in the CNS after ADX, most notable of which is elevated CRF production (15, 30).

CRF, in addition to mediating corticosterone secretion, has been implicated in SNS regulation. A subset of hypothalamic CRF neurons project to sympathetic preganglionic neurons in the spinal cord (16), and central injection of CRF increases various indexes of sympathetic activity (5, 14). The potential for interaction between the HPA axis and the SNS arises in part, from the inverse relationship between glucocorticoids and CRF release. Specifically, elevations of glucocorticoid levels inhibit CRF release (25), whereas ADX enhances CRF production and release (15, 30). One could hypothesize that ADX would enhance the effects of LPS on the SNS by making the central pathways that mediate...
sympathetic outflow more responsive to CRF-dependent, LPS-induced signals. Therefore, we have tested the effects of ADX on splenic and renal sympathetic nerve activity in response to peripheral (intravenous) injection of LPS. Possible effects of ADX on the responsiveness of the central immunoregulatory system were examined by means of central (intracerebroventricular) injection of PGE2 to test if the SNS and HPA axis interact at both central and peripheral levels. The present study utilized an acute ADX procedure to avoid the consequences of chronic ADX, including changes in constitutive neuropeptide expression (15, 30).

METHODS

Animals. Male Sprague-Dawley rats (300-400 g; Charles River, St. Constant, Quebec, Canada) were individually housed with free access to food and water under a 12:12-h light-dark cycle.

Electrophysiology. Under urethane-chloralose anesthesia (912.78 mg/kg), animals were fitted with tracheal tubes, and the femoral artery and vein were catheterized. A pressure transducer in the arterial catheter was used to monitor blood pressure (BP). Heart rate (HR) was detected from the arterial pulse pressure signal. A 1-ml/h infusion of anesthetic-saline (1:3) was given to maintain fluid and anesthesia levels. The spleen was accessed and dissected anteriorly to reveal the splenic neurovascular network. A peripheral branch of the splenic nerve was dissected free and placed on a bipolar platinum wire electrode. The preparation was insulated from the surrounding tissue with warm mineral oil and then embedded in silicone (Kwik-Cast; WPI, Sarasota, FL). Similarly, the left kidney was also dissected anteriorly to permit dissection of a branch of the renal nerve. The distal end of the renal nerve was pinched to eliminate any afferent impulses. The rat splenic nerve has been shown previously to consist exclusively of efferent sympathetic fibers (23). Electrical signals from the nerves were amplified, bandpass filtered (30–3,000 Hz), and displayed on an oscilloscope. Nerve impulses sufficiently above background noise were detected with a window discriminator, and the suprathreshold impulses were quantified by a rate meter (counts/5 s). The filtered nerve signal, rate meter output, BP, and HR were recorded on a strip chart recorder and digitally archived on videotape.

Acute adrenalectomy. Before femoral artery and vein catheter insertion, the right adrenal gland was dissected free from surrounding tissue, and the vasculature supplying the gland was securely occluded. The entire gland was then removed with little or no blood loss. The animal was then repositioned, and catheters were inserted. At this time, the second (left) adrenal gland was removed, and the preparation for nerve recording was completed. For experiments in which nerve recordings were obtained before and after acute ADX, only the right adrenal gland was removed as above. The animal was then prepared for sympathetic nerve recording, and baseline nerve activity was monitored for 30 min after which the second adrenal gland was removed while continuing to record nerve activity. Post-ADX sympathetic nerve activity was monitored for 45–60 min. Splenic and renal nerve impulses were recorded simultaneously during these recordings.

Intracerebroventricular cannulation. Rats were anesthetized with Somnotol (pentobarbital sodium, 60 mg/kg; MTC Pharmaceuticals, Cambridge, Ontario) and were placed in a stereotaxic apparatus. Stainless steel cannulas were implanted in the left ventricle using the following coordinates relative to bregma: posterior 0.8 mm, lateral 1.4 mm, ventrol 4.9 mm. Cannulas were fixed to the skull using stainless steel screws and dental cement. Animals were allowed to recover from surgery a minimum of 6 days before further experimental manipulation.

Plasma corticosterone assay. Separate groups of rats were used to determine plasma corticosterone levels in intact and ADX animals. These animals underwent similar surgical preparation as typically used for electrophysiological recordings without the actual placement of electrodes. To match the time course of events and the body positioning limitations that occur in the electrophysiology experiments, the ADX animals had one adrenal gland removed and were then repositioned, and an arterial catheter was inserted to permit the collection of blood samples. Approximately 40 min later, when all animals were prepared, a baseline sample was obtained from all animals after which the second adrenal gland was removed. Other groups of animals received LPS (10 µg) or saline at this time. Four additional samples were obtained at hourly intervals. Blood was collected into EDTA-treated tubes and held on ice until centrifuged, after which the plasma was stored at −20°C until assayed. Plasma corticosterone levels were determined using a standard RIA procedure. Briefly, samples and corticosterone standards were thawed and diluted in assay buffer, and aliquots were incubated on ice with anti-corticosterone antisemur and radioactively labeled corticosterone for 90 min. Bound and free components were separated by centrifugation after the addition of a charcoal-dextran mixture. The radioactivity of the supernatants was measured in a scintillation counter to determine the concentration of the unknown samples relative to the standards.

Experimental protocol. Nerve activity, BP, and HR were monitored for a minimum of 30 min to ensure a stable preparation had been obtained. Animals were then injected intravenously with LPS (Escherichia coli, 055:B5; Sigma) at a dose of 10 µg in 200 µl of saline and were monitored for ~90 min (non-ADX splenic nerve, n = 8; non-ADX renal nerve, n = 4; ADX splenic nerve, n = 7; ADX renal nerve, n = 6). In separate experiments, animals were injected intracerebroventricularly with 2 µg of PGE2 (Caymen Chemical) in 10 µl of saline (non-ADX splenic nerve, n = 5; non-ADX renal nerve, n = 6; ADX splenic nerve, n = 5; ADX renal nerve, n = 6). A final set of animals was pretreated intracerebroventricularly with a CRF antagonist, [−Phe12, Nle12,18,22-Cα-MeLeu23]CRF-(12-41) (4 µg in 10 µl saline; Peninsula Laboratories; see Ref. 21), or vehicle 20 min before intracerebroventricular injection of 2 µg of PGE2 (n = 3/group). In this last set of animals, the splenic and renal nerves were recorded simultaneously, whereas the splenic and renal nerves were recorded in separate animals for other experiments. A detectable increase in nerve activity was defined as a sustained increase of >15% above the baseline level and was expressed as percent increase above baseline. The time of onset was taken as the point at which the sustained increase occurred. The BP data were taken from data recordings at the point that corresponded to the peak response in nerve activity.

Statistical analysis. A two-way ANOVA was used to assess the main effects of adrenalectomy and nerve on the percent increase and onset latency after either 10 µg LPS intravenously or 2 µg PGE2 intracerebroventricularly. The effects of ADX on the systolic and diastolic BP responses to LPS or PGE2 were assessed by two-way ANOVA using systolic or diastolic BP before and after LPS or PGE2 injection as a repeated measure. A similar statistical design (2-way ANOVA) was also used for analysis of the nerve activity data obtained from simultaneous recordings of splenic and renal nerves in...
ADX rats with and without the CRF antagonist (antagonist vs. vehicle: between-groups factor, splenic vs. renal: repeated factor). In all cases, specific orthogonal contrasts were used to determine significant group differences (α = 0.05). Plasma corticosterone values were compared using a repeated-measures ANOVA with subsequent contrasts to determine significant group differences at each time point. All values are expressed as means ± SE.

RESULTS

Injection of 10 µg LPS intravenously increased both splenic and renal sympathetic nerve activity, with a more rapid response occurring for the splenic nerve (Fig. 1). Acute ADX was chosen to assess the role of circulating corticosterone on the sympathetic response to LPS to avoid the adaptive processes that occur after chronic ADX. Before electrophysiological experiments, the effect of acute ADX on plasma corticosterone was determined. Surgical preparation alone was a strong stimulus for corticosterone release, which was maintained throughout the observation period, and these levels were not increased further by injection of 10 µg LPS intravenously (Fig. 2). Acute ADX resulted in rapid dissipation of circulating corticosterone within the first hour. On the basis of these data, all further experiments involving ADX were performed no sooner than 1 h after removal of the adrenal glands.

Injection of 10 µg LPS after acute ADX produced a dramatic increase in splenic and renal nerve discharge that was significantly larger than the response observed in rats with intact adrenal glands (Fig. 1). Additionally, the onset latency of increased renal nerve activity was reduced in acute ADX animals (Fig. 1). BP in non-ADX rats was not altered by LPS, whereas both systolic and diastolic BP dropped after LPS injection in ADX rats (Fig. 3). Therefore, the hypotension induced by LPS in ADX rats likely contributed to the enhancement of sympathetic nerve responses.

Because such factors as hypotension and increased peripheral mediator production may result in greater nerve responses in ADX animals, a central stimulus was used to test whether the central sympathetic pathway was more responsive after acute ADX. For this, PGE₂, a potent stimulant of sympathetic activity, was injected directly into the CNS. In control animals, intracerebroventricular injection of PGE₂ induced an immediate increase in sympathetic nerve activity that was larger and occurred more rapidly for the renal nerve (Fig. 4). The PGE₂-induced splenic nerve response was not affected by ADX, although the onset latency was reduced. Thus increased CNS sensitivity to this LPS intermediary product does not account for the increased splenic nerve response to intravenous LPS in ADX animals. Unexpectedly, the response of the renal nerve to central PGE₂ was blunted in ADX animals (Fig. 4). This abrogated the significant difference between splenic and renal nerve responses to PGE₂, which was present in animals with intact adrenal glands.

It is possible that the reduced renal nerve response to intracerebroventricular PGE₂ was due to an increase in

---

1 These experiments represent ongoing studies, and, as such, control data (10 µg LPS) have been reported previously (18). Note: all experiments performed with LPS from the same lot number and within a 6-mo period.
baseline activity after ADX. An elevated baseline may have resulted in a proportionally smaller increase in nerve activity since responses are calculated relative to baseline activity. This concern was addressed by recording the splenic and renal nerves during the acute ADX. The percent change from baseline to 15 and 45 min after removal of the second adrenal gland was as follows: splenic (+15 min) −2.8 ± 5.9%, splenic (+45 min) 3.1 ± 1.3%, renal (+15 min) −1.5 ± 5.7%, and renal (+45 min) −0.5 ± 9.7% (means ± SE). Thus there were no significant main effects of nerve or time (P = 0.895 and 0.402, respectively), indicating that decreased renal nerve responses to intracerebroventricular PGE₂ were not due to altered baseline activity.

Diminished renal nerve responses in ADX rats may also have resulted from larger PGE₂-induced BP responses. The subsequent increase in baroreceptor feedback may have masked an otherwise normal PGE₂ response. However, the increase in BP brought on by intracerebroventricular PGE₂ was similar in control and ADX animals (Fig. 5). A minor trend (nonsignificant) toward reduced baseline BP was present, as might be expected after loss of this major source of catecholamines.

One outcome of high circulating levels of corticosterone is the inhibition of CRF release, and acute ADX would be expected to remove this inhibitory effect. Therefore, a CRF receptor antagonist was injected centrally to assess if enhanced CRF release was a factor in altering the renal nerve response to PGE₂ in ADX animals. In these experiments, the splenic and renal nerves were recorded simultaneously in the same animals. PGE₂ failed to induce a greater response from the renal nerve compared with the splenic nerve in vehicle-pretreated acute ADX animals (Fig. 6). Importantly, the attenuated renal nerve response in acute ADX animals obtained from averaging data from individual animals (Fig. 4) was reproduced in animals in which the nerves were recorded simultaneously (Fig. 6). In contrast, animals receiving intracerebroventricular injection of [α-Phe₁², Nle₂³⁸, Cα-MeLeu₃₇]CRF-(12—14) displayed a significantly greater response to intracerebroventricular PGE₂ from the renal nerve compared with that of the splenic nerve (Fig. 6). As a result, the response pattern between the nerves in ADX animals was restored by the CRF antagonist to that seen in adrenal-intact animals (Fig. 4).

**DISCUSSION**

The present set of experiments found that LPS-induced SNS responses were enhanced by acute ADX. This outcome may not be surprising since other LPS-induced responses, including the development of hypertension (9, 20) and the increase in circulating levels of cytokines (26), are exaggerated after ADX. However, we are not aware of any reports that have clearly demonstrated this effect within the SNS. For example, previous studies have not observed greater increases in plasma norepinephrine levels in ADX or adrenal demedullated animals relative to intact controls in response to LPS (9, 20). These apparent negative findings for plasma norepinephrine may be a result of alterations in both release from nerve terminals and clearance from
Acute ADX and Sympathetic Nerve Activity

Figure 5. Systolic and diastolic blood pressure in non-ADX and acute ADX rats before and after i.c.v. injection of 2 µg PGE₂ (n = 10/group). Values are expressed as means ± SE. Main effects for systolic blood pressure: adrenal status F(1,19) = 0.01, P = 0.944, time F(1,19) = 62.45, P < 0.001. Main effects for diastolic blood pressure: adrenal status F(1,19) = 0.01, P = 0.936, time F(1,19) = 50.8, P < 0.001.

*Significantly different from pre-PGE₂ data within adrenal condition, P < 0.05.

Enhanced sympathetic responses to LPS in ADX rats could be predicted based on reports that peripheral or central injection of cytokines induced by LPS increases sympathetic nerve activity (14, 24, 33), and ADX augments plasma and brain levels of cytokine production after LPS stimulation (12, 26). Therefore, the enhancement of sympathetic nerve responses by ADX can be explained by increased production of peripheral and/or central cytokine intermediates. In addition, the synthesis of other intermediary factors may have been enhanced by ADX. Central injection of PGE₂ rapidly increases sympathetic nerve activity (2, 19), and central inhibition of PG synthesis blocks the sympathetic response after peripheral injection of LPS (19). Glucocorticoids suppress basal and LPS-stimulated PG synthesis in the rat brain, and chemical or surgical ADX enhances LPS-induced PGE₂ synthesis (34). Therefore, enhanced production of PG may also contribute to the increased sympathetic nerve responses to LPS in ADX animals. Finally, baroreceptor mechanisms may have also contributed to the greater sympathetic responses seen in LPS-treated ADX rats given that BP decreased after LPS injection in ADX rats but not in adrenal-intact animals.

LPS also induces release of the neuropeptide CRF (11), which is involved in regulation of the HPA axis and the SNS (5, 6). CRF mRNA, protein, and release into the hypophysial portal blood system are all elevated by chronic ADX and, for the most part, normalized by glucocorticoid replacement (15, 30). Increased CRF gene transcription occurs 15–30 min after chemical ADX and is accompanied by elevated plasma levels of ACTH, which suggests that enhanced CRF release also occurs within this time frame (13). After acute ADX, CRF-dependent responses stimulated by LPS challenge (e.g., SNS and HPA activation) may be facilitated due to removal of the negative feedback signal of corticosterone on CRF release. To assess whether the CNS was more responsive to LPS-related stimuli after acute ADX, we injected PGE₂ directly into the lateral cerebral ventricle, a procedure known to increase sympathetic outflow (19). Injection of PGE₂ rapidly increased sympathetic discharge of both nerves, with a preferential effect on the renal nerve. The magnitude of the splenic nerve response to PGE₂ was not affected by acute ADX, indicating that sensitivity of central pathways regulating sympathetic outflow to the spleen was not affected by ADX or any putative changes in CRF regulation. These data suggest that, in ADX animals, the increased sympathetic responses induced by LPS arise from changes in intermediary factor production and baroreceptor feedback rather than enhanced sensitivity of the autonomic centers in the CNS to the LPS stimulus.

Despite increased responsiveness of the renal nerve to LPS in ADX rats, PGE₂ was less effective in stimulating renal nerve activity after ADX. The reduced effect of PGE₂ on renal nerve activity in ADX animals can potentially be explained by a shift in the baseline activity of the nerve. Chronic ADX is known to enhance basal norepinephrine release (4); therefore, the ADX by itself may have increased basal renal nerve activity, resulting in a smaller relative increase after PGE₂ injection. In other words, inducing an absolute response (PGE₂) on top of a relatively larger baseline would give the appearance of reduced activation. To address this possibility, we recorded splenic and renal sympathetic nerve activity during the ADX procedure. Although one adrenal gland had already been removed due to positioning requirements for the electrophysiological recordings, plasma corticosterone levels were main-
tained at \( \sim 70\% \) of the control animals and fell rapidly after removal of the second adrenal gland (Fig. 2). Despite this marked decline in plasma corticosterone levels, no effects were observed on renal or splenic nerve activity after removal of the second adrenal gland. These experiments were designed to reproduce the same time course of events as occurred in the electrophysiological experiments (i.e., the interval between ADX and LPS or PGE2 injection). Thus sympathetic discharge is not enhanced immediately upon ADX, although basal sympathetic activity is increased by 24 h after ADX (4).

Baroreceptor-mediated reflexes are another possible mechanism of inhibited renal nerve responses to intracerebroventricular injection of PGE2 in ADX rats. If PGE2 induced greater increases in BP after ADX, then the negative feedback from the baroreceptors should also be greater. In turn, the activity of the sympathetic nerves may be masked by this inhibition. However, BP before or after injection of PGE2 was not altered by ADX, making it unlikely that pressor-related factors are responsible for the changes in renal nerve activity.

Glucocorticoids inhibit, whereas ADX enhances, CRF release from parvocellular neurosecretory neurons (6, 25). The increase in CRF release appears to begin almost immediately, since plasma ACTH levels are rapidly elevated after surgical ADX (7). Similar effects have been reported after adrenal-intact animals (13). Although ADX does not appear to increase CRF immunoreactivity in parvocellular cells with descending projections (29), ADX effects could still be mediated by other neural circuits that are sensitive to corticosterone/CRF and provide inputs to autonomic centers. One such area is the dorsomedial nucleus of the hypothalamus, which has dense inputs to parvocellular neurons with descending projections (31) and which expresses both glucocorticoid and CRF receptors (1, 27). To assess if enhanced CRF release was involved in the altered renal nerve response to PGE2 after acute ADX, we pretreated ADX animals with a potent antagonist of CRF, [d-Phe12, Nle21,38,Co-MeLeu37]-CRF-(12—41) (21), and recorded splenic and renal nerve activity after PGE2 injection. Consistent with the earlier manipulation of the HPA axis, the splenic nerve response was also unaffected by pretreatment with the CRF antagonist. In contrast, the CRF antagonist significantly enhanced the increase in renal nerve activity after PGE2 injection. Thus manipulations that should have resulted in greater CRF release caused an inhibition of the PGE2-induced renal sympathetic nerve response. This effect was reversed with a CRF antagonist such that the renal nerve response was once again greater than that of the splenic nerve, as was the case in adrenal-intact animals. Although a higher dose of antagonist may have had an effect on the splenic nerve response to PGE2, the dose used was effective regarding the renal nerve.

The actions of the CRF antagonist were consistent for all animals and produced a statistically significant effect despite the relatively small group size (likely due to the greater statistical power of the repeated-measures design used in this experiment).

Two of the experiments described here were specifically designed to assess if manipulations that should have altered CRF release were able to modify sympathetic nerve responses to central stimulation. The data indicate that CRF and the HPA axis are not directly involved in mediating sympathetic outflow to the spleen after intracerebroventricular injection of PGE2. In addition, these data suggest that renal sympathetic nerve activity may be inversely related to CRF release. Other investigators have reported contrary findings in that central injection of a CRF antagonist blocked the increase in splenic nerve activity after PGE2 injection and that central injection of CRF increased splenic nerve activity (14, 17). The discrepancy regarding the actions of a CRF antagonist on splenic nerve activity induced by PGE2 may be due to the adrenal status of the animals. In the present study, the CRF antagonist was only injected in acute ADX rats, whereas previous studies used adrenal-intact animals (17). Another possibility is the difference in the CRF receptor antagonists [d-Phe12, Nle21,38,Co-MeLeu37]-CRF-(12—41) vs. \( \alpha \)-helical CRF-(9—41); see Ref. 17. In addition, much higher antagonist-to-agonist ratios have been used previously, whereas a 2:1 ratio was used in the present study. Despite the low 2:1 ratio, we observed significant effects of the CRF antagonist on the renal nerve response to PGE2. Finally, some differential effects may have arisen from the type of nerve fibers recorded. Because the rat splenic nerve consists solely of efferent sympathetic fibers (23), whole nerve recordings were used in the present study in contrast to a subset of splenic nerve fibers preselected for displaying baroreceptor sensitivity (17).

Other experiments measuring plasma catecholamines, HR, and BP in conscious animals also found an increase in SNS activity after CRF injection (5). However, CRF also stimulates increases in behavioral activity (8), and it is unclear to what extent the autonomic outcome measures are stimulated by the locomotor effects of CRF injection. In addition, intracerebroventricular injection of CRF inhibits parasympathetic activity, which contributes to the CRF-induced tachycardia (10). Thus it is not clear whether the sympathetic activation attributed to CRF results from direct action on central pathways that mediate sympathetic outflow or through effects on other brain areas that then impact on sympathetic pathways (e.g., vagal centers). The ineffectiveness of the CRF antagonist in blocking PGE2-mediated sympathetic stimulation suggests that CRF may not be directly involved in the central pathways mediating sympathetic activity. Consistent with the present data, other investigators have reported that PGE2-mediated pyrogenic and metabolic responses are not affected by a CRF antagonist (28).

Although the magnitude of the splenic nerve response to PGE2 was not altered by ADX, there was a significant reduction in onset time. This effect was not reversed by pretreatment with the CRF antagonist, which may indicate that a glucocorticoid-sensitive, but...
not CRF-related, process was responsible for this outcome. However, a wider range of doses for the CRF antagonist would be required before a more definitive statement could be made regarding this possibility.

In summary, peripheral injection of LPS and central injection of PGE\textsubscript{2} increased the electrical activity of the splenic and renal sympathetic nerves. Acute ADX enhanced the response of both nerves to LPS. In contrast, the splenic nerve response to PGE\textsubscript{2} was not affected by acute ADX, whereas the renal nerve response was reduced in ADX animals. This outcome was not due to the effects of acute ADX itself on sympathetic nerve activity or to differences in BP responses in ADX vs. control animals. Injection of a CRF antagonist restored the PGE\textsubscript{2}-mediated renal nerve response to that observed in non-ADX animals. The splenic nerve response to PGE\textsubscript{2} was unaffected by the CRF antagonist. These data indicate that the enhanced sympathetic activation induced by LPS in acute ADX rats was not mediated by increased responsiveness of the central sympathetic pathways. In addition, CRF does not appear to play a direct role in mediating sympathetic outflow to the spleen induced by central injection of PGE\textsubscript{2}.

Perspectives

The SNS is able to modify both innate and acquired parameters of immune function. However, much of this research has been carried out using in vitro preparations, and, as a result, less is known regarding the precise in vivo function of sympathetic innervation of immune organs. If the SNS is to contribute to the regulation of immune function during typical immune challenges, then a definable neuroanatomic and neurochemical pathway is required by which this regulatory message can be conveyed to immune organs. In particular, a collection of neurons within the SNS must be able to transmit immune-related impulses to immune organs independent of other autonomic functions, such as maintenance of peripheral vascular tone. Otherwise, any and all episodes of sympathetic activation would have the potential of modifying immune function. To this end, we have undertaken studies that test the critical hypothesis that effective sympathetic regulation of immune function necessitates a definable sympathetic-immune pathway capable of functioning in a target-specific manner. The present study demonstrates that the splenic and renal sympathetic nerves in the rat are capable of differential response patterns upon exposure to immune-related stimuli. Future studies will continue to investigate the precise neuroanatomic and neurochemical mechanisms that may mediate regulatory sympathetic information to immune cells.

This research supported by the National Institute of Mental Health Grant MH-43778-04A2.

Address for reprint requests and other correspondence: D. M. Nance, Dept. of Pathology, Univ. of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3 (E-mail: dnance@csrc.umanitoba.ca)

Received 19 July 1999; accepted in final form 8 December 1999.

REFERENCES

unrestrained rats: effects of adrenal demedullation and/or gua-


22. Munck A and Guyre PM. Glucocorticoids and immune func-


25. Plotsky PM and Vale W. Hemorrhage-induced secretion of corticotropin-releasing factor-like immunoreactivity into the rat hypophysial portal circulation and its inhibition by glucocorti-


28. Rothwell NJ. Central activation of thermogenesis by prosta-


30. Sawchenko PE. Adrenalectomy-induced enhancement of CRF and vasopressin immunoreactivity in parvocellular neurosecre-
tory neurons: anatomic, peptide, and steroid specificity. J Neuro-

31. Ter Horst GJ and Luiten PGM. Phasedus vulgaris leuco-
agglutinin tracing of intrahypothalamic connections of the lat-
eral, ventromedial, dorsomedial and paraventricular hypothal-

32. Turnbull AV, Lee S, and Rivier C. Mechanisms of hypotha-

33. Vriend CY, Zuo L, Dyck DG, Nance DM, and Greenberg AH. Central administration of interleukin-1β increases norepineph-