Peripheral corticotropin-releasing factor mediates the elevation of plasma IL-6 by immobilization stress in rats

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Ando, Tetsuya, Jean Rivier, Hitoshi Yanaihara, and Akira Arimura. Peripheral corticotropin-releasing factor mediates the elevation of plasma IL-6 by immobilization stress in rats. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1461–R1467, 1998.—We previously reported the elevation of plasma interleukin (IL)-6 activity in response to immobilization stress in rats. To investigate the role of peripheral corticotropin-releasing factor (CRF) in this response, we examined the effects of CRF antagonists on immobilization-induced IL-6 response. Intravenous pretreatment with either [D-Phe12,Nle21,38,C=MeLeu37]-anti-human rat (h/r)CRF 12—41 (1.5 mg/kg) or cyclo(30—33)[D-Phe12,Nle21,38,Glu30,Lys33]-h/rCRF 12—41 (Astressin, 0.5 mg/kg) attenuated the IL-6 response to immobilization, which confirmed our previous finding that systemic administration of an antisera against CRF blocked this response. In addition, an intraperitoneal injection of h/rCRF (100 µg/kg) or rat urocortin (10 and 100 µg/kg) increased the plasma IL-6 activity, mimicking the response to immobilization. An intravenous injection of h/rCRF (100 µg/kg) also elevated plasma IL-6 in adrenalectomized rats. These findings suggest that peripheral CRF mediates the plasma IL-6 elevation in response to immobilization.

INTERLEUKIN (IL)-6 was originally identified as a soluble immune tissue-derived factor that promotes the proliferation and differentiation of lymphocytes (27). Many reports have detailed the pleiotropic actions of IL-6, such as its induction of acute phase protein synthesis in the liver (15), stimulation of the hypothalamic-pituitary-adrenal (HPA) axis (21), cytoprotective action against lethal irradiation (22), and neurotropic actions (10). These pleiotropic activities appear to play important roles in the host defense mechanism (2).

The elevation of plasma IL-6 activity in response to noninflammatory or noninfectious stressors such as restraint (29, 36), electrical foot shock (36), and exposure to open field (16) has been reported. We have investigated the mechanisms of this stress-induced plasma IL-6 response using immobilization as a stressor (3, 29). Chemical depletion of central catecholamine with intracerebroventricular administration of 6-hydroxydopamine (6-OHDA) attenuated immobilization-induced plasma IL-6 response. Depletion of peripheral norepinephrine with intravenous 6-OHDA also reduced the response; thus both the central and peripheral catecholaminergic system appear to be involved (29). The HPA axis may exhibit an inhibitory influence, because both hypophysectomy and adrenalectomy augmented the immobilization-induced IL-6 response (29).

Corticotropin-releasing factor (CRF), a 41-amino-acid peptide, was first identified as a hypothalamic factor that stimulates the secretion of ACTH from the anterior pituitary (31, 34). CRF not only acts as a hypophysiotropic hormone but also functions as a neurotransmitter or neuromodulator (4). There is abundant evidence that CRF mediates integrated endocrine and autonomic and behavioral responses to stress (8, 24). Our previous studies revealed that intravenous pretreatment with a rabbit antiserum against CRF significantly suppressed both ACTH and IL-6 responses to immobilization (3). Intracerebroventricular injection of a CRF antagonist (α-helical CRF) attenuated the ACTH response but failed to suppress the IL-6 response to immobilization (3). These findings suggest that peripheral CRF, rather than central CRF, is involved in the immobilization-induced plasma IL-6 response.

The existence of a CRF-like immunoreactivity or a CRF-like substance has been reported in many peripheral tissues (1, 20, 23, 25, 33). A novel intrinsic CRF-related peptide, urocortin, has been cloned from rat brain (35) and human placenta (7). Urocortin has a higher affinity and activity than CRF on type 2 CRF receptors, which are the dominant subtype in peripheral tissues, suggesting its role as a natural ligand for type 2 CRF receptors in peripheral tissue (35). Although the physiological role of peripheral CRF or CRF-like substances remains unknown, its local effects on immune or inflammatory processes have been suggested (5, 12, 17).

Recently, new CRF antagonists, [D-Phe12,Nle21,38,C=MeLeu37]-anti-human rat (h/r)CRF 12—41 (α-helical CRF) and cyclo(30—33)[D-Phe12,Nle21,38,Glu30,Lys33]-h/rCRF 12—41 (Astressin) have been developed (6, 9, 11). Because these new antagonists possess a high affinity for CRF receptors, a high solubility to water, and a relatively low affinity to CRF binding protein, they are more potent in blocking ACTH secretion after peripheral administration in vivo than previous antagonists such as α-helical CRF (6, 9, 11).

In the present study, we examined whether systemic pretreatment with CRF antagonists blocks immobilization-induced IL-6 response. We also tested the effects of intraperitoneal injections of various doses of CRF and urocortin on plasma IL-6 levels. To eliminate the suppressive influence of the HPA axis, we examined the effect of an intravenous injection of CRF using adrenalectomized rats.
METHODS

Animals. Male CD rats (Charles River, Wilmington, MA) weighing 350–400 g were used. The animals were housed in a room maintained at ~25°C with illumination from 0800 to 2000. Standard Purina laboratory chow and water were available ad libitum. The experimental protocol was approved by the Tulane University Medical School Advisory Committee for Animal Resources (protocol no. 1783–3–03–105).

Cannulation into the jugular vein. To collect blood samples for plasma IL-6 and ACTH measurement, we implanted the animals with an indwelling jugular venous catheter filled with heparinized saline solution (50 U/ml). Surgery was performed 2 days before the experiment under ketamine (100 mg/kg ip) and xylazine (10 mg/kg ip) anesthesia.

Immobilization stress. The animals were adapted to the experimental conditions by daily handling for 7 days to avoid manipulative stress. On the day of the experiment, the cages were removed from the home cages and the rats were restrained on a board in a supine position by taping the limbs to holders for 60 min without anesthesia.

Blood sampling. Blood samples (0.7 ml) for determination of plasma IL-6 and ACTH levels were taken through the jugular vein catheter 10 times (at ~30, 0, 15, 30, 60, 90, 120, 180, 240, and 300 min) before, during, and after immobilization stress. Each sample was collected in an ice-chilled tube containing Trasylol (aprotinin; 500 KIU, Mobay), and EDTA buffer saline, pH 7.4, containing 0.1% bovine serum albumin and 0.01% ascorbic acid. The injection volume was 1 ml for both the intravenous and intraperitoneal injections.

Plasma IL-6 and ACTH responses to immobilization. Figure 1A shows the plasma IL-6 response to 1-h immobilization. The values of each group were averaged and plotted against the individual times. Basal levels of plasma IL-6 were 21.8 ± 4.3 U/ml in the immobilized group and 30.3 ± 7.5 U/ml in the non-stressed control group; there was no significant difference between the two. Plasma IL-6 levels started to increase 30 min after the initiation of the immobilization, peaked at 90 min (1,989.6 ± 507.5 U/ml), quickly dropped at 120 min, and then gradually returned to the basal level after more than 300 min. There was a significant interaction effect of the immobilization and time over plasma IL-6 activity (two-way ANOVA, split-plot design, F = 2.288, P = 0.0022). The levels of IL-6 in the immobilized animals at several time points were significantly higher than the basal level [60 and 90 min, repeated-measures ANOVA (F = 11.917, P = 0.0076) followed by Dunn's t-test, P < 0.01] or those in the control animals (30, 90, 120, 180, and 300 min, Student's t-test, P < 0.005). There was no significant change of IL-6 activity in the control animals over time (F = 2.288, P = 0.1364).

Figure 1B shows plasma ACTH levels immediately before immobilization and at 15 min after the initiation of the stress. There was a significant interaction effect of the immobilization and time over plasma ACTH concentration (F = 16.574, P = 0.0022). Immobilization increased plasma ACTH concentrations at 15 min significantly as compared with the basal (P < 0.01) and the control (P < 0.01).
Blockade of plasma IL-6 and ACTH response to immobilization by CRF antagonists. To investigate the possible involvement of peripheral CRF in immobilization-induced plasma IL-6 elevation, a CRF antagonist, either D-Phe-CRF12–41 (1.5 mg/kg) or Astressin (0.5 mg/kg), was injected intravenously 5 min before the initiation of immobilization. Pretreatment with either D-Phe-CRF12–41 (Fig. 2A) or Astressin (Fig. 3A) significantly attenuated the plasma IL-6 responses to immobilization compared with the vehicle (F = 5.965, P = 0.0156, and F = 3.680, P = 0.0329, respectively). Comparison at individual time points revealed that the IL-6 level at 90 min in the D-Phe-CRF12–41-treated group was significantly lower than that in the vehicle group (254.9 ± 81.4 vs. 1,512.7 ± 238.1 U/ml, Student’s t-test, P = 0.0011). Astressin also reduced the average of peak IL-6 levels at 90 min (1,032.2 ± 151.6 vs. 2,104.7 ± 388.1 U/ml) (P = 0.0244).

Both antagonists attenuated immobilization-induced plasma ACTH elevation (D-Phe-CRF12–41: F = 29.466, P = 0.0006; Astressin: F = 14.435, P = 0.0029) (Figs. 2B and 3B). The ACTH concentration at 15 min in either the D-Phe-CRF12–41- or Astressin-treated group was significantly lower than that in corresponding vehicle control (P < 0.05).

Plasma IL-6 response to intraperitoneal injections of CRF and urocortin. To further investigate a possible involvement of peripheral CRF in the immobilization-induced IL-6 elevation, either rat CRF (10 and 100 µg/kg) or rat urocortin (10 and 100 µg/kg) was injected intraperitoneally and we examined the changes in plasma IL-6 and ACTH concentrations.

An intraperitoneal injection of CRF increased plasma IL-6 in a dose-dependent manner (Fig. 4). The peak effect was observed at 90 min (173.0 ± 50.6 U/ml at 10 µg/kg and 626.4 ± 142.4 U/ml at 100 µg/kg) after the injection. The IL-6 concentration returned to baseline levels after more than 300 min. There was a significant interaction effect of CRF treatment and time over plasma IL-6 activity (F = 7.547, P = 0.0002). CRF at 100 µg/kg increased IL-6 activity significantly compared with the basal level (F = 3.669, P = 0.1019, and F = 2.891, P = 0.1386, respectively). CRF at 100 µg/kg differed from the vehicle control at 90 and 120 min (1-way ANOVA, P < 0.005 followed by Dunn’s test, P < 0.05).

An intraperitoneal injection of urocortin, a CRF-related peptide, also increased plasma IL-6 significantly (F = 3.27, P = 0.0471), peaking at 120 min...
Urocortin at 10 and 100 µg/kg increased plasma IL-6 activity significantly compared with the basal level (F = 6.368, P = 0.0470, and F = 7.754, P = 0.0398, respectively) (Fig. 5A).

An intraperitoneal injection of either CRF or urocortin elevated the plasma ACTH concentration significantly (F = 6.177, P = 0.0143, and F = 13.598, P = 0.0008, respectively). Either CRF or urocortin at 100 µg/kg increased ACTH levels significantly from the basal levels or the vehicle at 30 min (P < 0.05). At 10 µg/kg, ACTH levels remained unchanged (P > 0.05).

Plasma IL-6 response to intravenous injections of CRF in adrenalectomized animals. In contrast to intraperitoneal injection, intravenous injection of CRF (100 µg/kg) does not cause significant changes in plasma IL-6 levels in intact rats (3). Intravenous injection of CRF quickly increases plasma ACTH and subsequently corticosterone levels, and high levels of corticosterone are known to suppress the IL-6 response to immobilization (29) and hemorrhagic shock (14). Thus, to eliminate the suppressive influence of the HPA axis, we examined the effect of an intravenous injection of CRF using adrenalectomized rats that had received corticosterone pellets subcutaneously to maintain normal levels of plasma corticosterone. Figure 6 shows the effect of intravenous injection of CRF (2–100 µg/kg) on plasma IL-6 levels. The basal corticosterone concentrations were 3.6 ± 0.9 to 4.0 ± 0.6 µg/dl, and there was no significant difference among groups.

There was a significant overall effect of intravenous CRF administration on plasma IL-6 activity (F = 4.142, P = 0.0214), although the interaction effect of the CRF treatment and time was not statistically significant (F = 2.341, P = 0.0941). The total mean of IL-6 activities in CRF 100 µg/kg group was significantly higher than that in the vehicle group (1,102.5 ± 283.8 vs. 56.2 ± 6.3 U/ml, P < 0.05; Fig. 6). However, lower doses of CRF (2 and 20 µg/kg) did not change the plasma IL-6 levels as compared with the vehicle control (P > 0.05).

DISCUSSION

The present data show that an intravenous pretreatment with a specific CRF receptor antagonist, D-Phe-CRF12–41 (1.5 mg/kg) or Astressin (0.5 mg/kg), significantly attenuates plasma IL-6 response to immobilization.
This is consistent with our previous finding that an intravenous injection of 0.5 ml rabbit antiserum to rat CRF suppressed the IL-6 response (3). The potent inhibiting action of these CRF antagonists on ACTH secretion in vivo has already been reported. A bolus intravenous pretreatment with 1.7 mg/kg D-Phe-CRF12—41 and 0.1 mg/kg Astressin inhibited ACTH secretion in the adrenalectomized rats for more than 60 and 90 min, respectively (9, 11). Intravenous pretreatment with Astressin at 0.3 mg/kg blocked electric shock-induced ACTH secretion for at least 30 min in rats (9). In the present study, treatments with these CRF antagonists also inhibited the ACTH response to 1 h immobilization, proving their effectiveness in blocking the actions of endogenous CRF induced by this stressor.

It is unlikely that the attenuation of IL-6 response was brought about by this ACTH blocking action, because either hypophysectomy or adrenalectomy enhances the IL-6 response to immobilization (29). Although it is not known whether peripherally administered CRF antagonists can suppress the action of CRF acting as a neurotransmitter/modulator in the brain, our previous studies showed that an intracerebroventricular injection of a CRF antagonist, α-helical CRF (25 µg over 5 min followed by 25 µg over 60 min), failed to block stress-induced IL-6 elevation (3). Thus the action of peripheral CRF, rather than the central CRF, seems essential for the immobilization-induced plasma IL-6 elevation. This is supported by the present findings that an intraperitoneal injection of either CRF (100 µg/kg) or urocortin (10–100 µg/kg) elevated plasma IL-6 levels with a time course similar to that observed after immobilization.

In contrast to an intraperitoneal injection, an intravenous injection of a high dose of CRF (a bolus injection of 50 µg/kg followed by an infusion of 50 µg/kg over 30 min) was not found to cause significant changes in plasma IL-6 levels in intact rats (3). The present study revealed that an intravenous administration of CRF did increase plasma IL-6 levels in adrenalectomized animals. Therefore, the ineffectiveness of intravenous CRF in normal rats might be attributed to the rapid activation of the HPA axis by CRF, which is known to suppress IL-6 production (14, 29). Another possible reason for the discrepancy of effectiveness between intravenous and intraperitoneal CRF is that the site of CRF action regarding the IL-6 response may be within the peritoneal cavity. In fact, the existence of CRF or CRF-like immunoreactivity has been reported in many abdominal organs, including the liver (28), pancreas (26), stomach (23, 33), and colon (13). Further study is necessary to determine the peripheral site of CRF action on IL-6 production and/or secretion during stress.

Our previous studies demonstrated that both central and peripheral catecholamines are involved in the immobilization-induced plasma IL-6 response (29). CRF has been detected in sympathetic ganglia in monkeys (32), as well as in neurons in spinal intermediolateral cell column in rats (20), and its role in modulating the release of catecholamine has been postulated (32). Therefore, it is possible that intraperitoneal injection of CRF stimulates peripheral sympathetic systems, thereby increasing plasma IL-6. However, the exact relationship between the sympathetic nervous system and the peripheral CRF in the stress-induced IL-6 elevation remains to be investigated.
The role of CRF as a local proinflammatory agent has also been suggested. Treatment with an anti-CRF antiserum suppressed carrageenin-induced inflammation, and a high concentration of CRF was detected in the inflamed tissue (12). CRF was present in the joints and surrounding tissues of Lewis rats with streptococcal cell-wall- and adjuvant-induced arthritis, and CRF mRNA was expressed in the inflamed synovia (5). CRF was also reported to stimulate leukocytes to secrete cytokines such as IL-1, -2, and -6 (17, 30).

CRF receptors are divided into two subtypes, designated type 1 and type 2 receptors. The type 2 receptor has two splice variants, type 2α and type 2β, with type 2β being the dominant subtype in peripheral tissues (4, 18). Neither Astressin nor D-Phe-CRF12—41 is selective for peripheral CRF receptors than CRF (35). We observed that urocortin at 10 µg/kg elevated plasma IL-6 activity significantly, whereas CRF at the same dose did not. This finding may be related to the different affinities of these agonists to peripheral CRF receptors.

The elevation of plasma IL-6 concentration caused by psychological stressors is considered to be one of the most valuable models for studying the brain's control over immune functions and host defense mechanisms. We conclude from the present study, together with our previous findings, that peripheral CRF is involved in stress-induced plasma IL-6 elevation. Further studies may reveal the underlying mechanisms and suggest the physiological and pathological roles of IL-6 elevation by stress.

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