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Evidence for a role of nitric oxide in the corticotropin-releasing factor release induced by interleukin-1β

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Abstract

Interleukin-1β stimulates corticotropin-releasing factor (CRF) secretion from the hypothalamus involving the activation of prostaglandins. This study investigated the possibility that nitric oxide (NO) acts as a mediator of interleukin-1-induced CRF release. An in vitro rat hypothalami continuous perifusion system was used. Pre- and co-incubation of hypothalami with either the NO synthase inhibitor, N^G-nitro-L-arginine (1 mM), or the NO scavenger, hemoglobin (10 μM), induced a marked reduction in the effect of interleukin-1 (3 pM) on CRF secretion. The effect of N^G-nitro-L-arginine was prevented by pre-exposure of hypothalami to L-arginine (1 mM). We also studied whether the involvement of NO in this interleukin-1 effect could involve a prostaglandin action. The concurrent treatment with N^G-nitro-L-arginine and indomethacin (14 μM) – an inhibitor of prostaglandin production – reduced interleukin-1-induced CRF release to the same level as N^G-nitro-L-arginine alone, suggesting that prostaglandins might interact with NO on this interleukin-1 effect. These results suggest that NO plays a role in the in vitro stimulatory action of interleukin-1 on hypothalamic CRF secretion.

Keywords: Interleukin-1; Nitric oxide (NO); CRF (corticotropin-releasing factor; Neuroimmunology; N^G-Nitro-L-arginine; Hemoglobin; Indomethacin; Prostaglandin

1. Introduction

Interleukin-1β, a cytokine released during infection and inflammation by activated macrophages and monocytes, exerts a wide array of effects in the nervous system (for a review see Rothwell and Dantzer, 1992). In addition, the production of interleukin-1 by glial and neural elements within the central nervous system (CNS) has now been demonstrated (Schultzberg, 1992). One of the best characterized actions of this cytokine is an important activating effect on the hypothalamic-pituitary-adrenal axis (Bateman et al., 1989; Rothwell and Dantzer, 1992). In particular, interleukin-1 stimulates hypothalamic secretion of corticotropin-releasing factor (CRF) (Berkenbosch et al., 1987; Sapolsky et al., 1987). Using an in vitro continuous perifusion system, we have previously observed a direct stimulatory action of interleukin-1 on hypothalamic CRF secretion, an effect which is under glucocorticoid modulation (Cambronero et al., 1989).

Nitric oxide (NO), a gas molecule which plays a wide array of roles in different physiological systems, has been identified as an intercellular messenger in the central and peripheral nervous systems (Bredt and Snyder, 1992; Garthwaite, 1991; Moncada et al., 1991). NO synthase is localized, among other brain areas, in the hypothalamic paraventricular nucleus (Bredt et al., 1990), the main locus of CRF synthesis. Interleukin-1 has been described to induce NO in blood (Kosaka et al., 1992) and astrocytes (Simmons and Murphy, 1993; Vigne et al., 1993) by inducing the synthesis of NO synthase, an enzyme that converts L-arginine into NO and L-citrulline (Moncada et al., 1991). In addition to this inducible form of NO synthase, a constitutive form has been located in vascular endothelium (Moncada et al., 1991) and the CNS (Bredt and Snyder, 1992). Evidence is accumulating for a role of NO in hormone and neurotransmitter release (Guevara-Guzman et al.,

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1994; Lutz-Bucher and Koch, 1994; Montague et al., 1994; Ota et al., 1993). Thus, a neuropeptide-releasing activity of NO at the hypothalamic level has been suggested, including vasopressin release (Ota et al., 1993) and acetylcholine- or interleukin-2-stimulated CRF secretion (Karanth et al., 1993).

NO has been shown to activate soluble guanylate cyclase, leading to increases in cyclic GMP levels (Garthwaite, 1991), and cyclooxygenase enzymes, resulting in an increase in prostaglandins (Salvemini et al., 1993). In particular, NO mediates the release of prostaglandin E_2 induced by noradrenaline in the hypothalamus (Rettori et al., 1992). The CRF- releasing activity of interleukin-1 in the hypothalamus has been reported to involve a prostaglandin action (Cambronero et al., 1992a; Navarra et al., 1991). Therefore, the possibility exists that the CRF secretory action of interleukin-1 might involve the production of NO either as an independent/parallel mechanism of prostaglandin activation or as a previous step mediating prostaglandin formation. In fact, NO has been shown to be involved in the hypothalamic CRF-stimulating action of another cytokine, interleukin-2 (Karanth et al., 1993). However, during the progress of the present work, a study (Costa et al., 1993) was published in which the in vitro stimulatory effect of interleukin-1 in a static incubation system did not appear to be dependent upon NO. Since the experimental conditions in this latter study differed from the continuous perifusion system we used, and since these different systems have previously proved to yield divergent results (Cambronero et al., 1992b; Navarra et al., 1991), we decided to further explore the possibility that interleukin-1 might release CRF via NO in our experimental model.

Therefore, the present work was designed to investigate the possible involvement of NO in the hypothalamic induction of CRF release by interleukin-1. We also evaluated whether NO could be a step in the chain of messengers, including prostaglandins, mediating this central secretory effect of the cytokine.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (250–300 g) from our in-house colony were used. They were housed four to five per cage under temperature (22 ± 2°C)- and light (12:12 light-dark cycle)-controlled conditions and had free access to food and water. The rats were decapitated between 10:00 and 11:00 a.m. After rapid removal of the brain, hypothalamic blocks were dissected with the following limits: posterior border of the optic chiasm, anterior border of the mammillary bodies, lateral hypothalamic sulci, and ventral border of the thalamus. The hypothalami (average weight = 20 mg) were then bisected by a longitudinal cut in the midsagittal plane.

2.2. Test materials

Human recombinant interleukin-1β (isoelectric point = 7.0, molecular weight = 17500, activity: 100 U/ng) was obtained from Genzyme (Cambridge, MA, USA). N^G-nitro-L-arginine, L-arginine, bovine hemoglobin, indomethacin, and veratridine were purchased from Sigma Co. (St. Louis, MO, USA). All compounds were dissolved immediately before use in the perifusion medium to reach their final concentration.

2.3. Rat hypothalamus continuous perifusion

The perifusion medium was Hanks' medium 199 (Gibco, Glasgow, Scotland) supplemented with 0.25% bovine serum albumin, 4 mM HCO_3Na, 6 g/l glucose and 5 mg/ml gentamicin, pH 7.35. The perifusion system consisted of a plastic syringe barrel connected by a pump-tubing to a peristaltic pump at one end and to an outflow-tube leading from the other end to a fraction collector. The tubing, the perifusion chambers and the media used were kept at 37°C by a circulating water bath. Fragments from five hypothalami were placed with Sephadex G-15 into a perifusion chamber, in a volume of 0.75 ml. The flow rate was 0.5 ml/min. After a stabilization period of 30 min, the releasing activity of human recombinant interleukin-1β in pulses of 10 min was tested. Interleukin-1 stimulates CRF secretion from superfused hypothalami in a dose-dependent manner within the range of 0.6–12 pM (Cambronero et al., 1989); we selected an intermediate active dose of interleukin-1 (3 pM) to carry out the following experiments. Hypothalami maintained in a continuous perifusion system were first subjected to an interleukin-1 pulse for 10 min. In the study of the effect of NO synthesis inhibition, hypothalami were perifused 40 min later with N^G-nitro-L-arginine for 20 min and then subjected to a second identical interleukin-1 pulse without stopping N^G-nitro-L-arginine infusion until the end of the interleukin-1 pulse. N^G-nitro-L-arginine, by competing with L-arginine for NO synthase, inhibits endothelial and brain NO production (Dwyer et al., 1991; Lambert et al., 1991). When the specificity of N^G-nitro-L-arginine effect was studied, L-arginine was infused 20 min prior to treatment with N^G-nitro-L-arginine and interleukin-1 (as above), with L-arginine infusion being maintained until the end of the other stimuli pulse. Further experiments tested the ability of the NO scavenger, hemoglobin, to interfere with interleukin-1-induced CRF secretion. A hemoglobin pulse was given 20 min before and during stimula-

tion of hypothalami with a 10-min interleukin-1 pulse. Forty min later, a second identical interleukin-1 pulse was given. Finally, a fourth series of experiments assessed possible interactions of NO with the eicosanoid cyclooxygenase pathway in the CRF response to interleukin-1. The cyclooxygenase inhibitor, indomethacin (14 μM), was previously shown to diminish the CRF-releasing activity of interleukin-1 (Cambronero et al., 1992a). Here, experiments were performed in which, after study of the effect of interleukin-1 alone (see above), hypothalami were exposed to the concurrent addition of indomethacin and N^G-nitro-L-arginine 20 min prior to and during a second 10-min interleukin-1 pulse. Eluate fractions (2.5 ml) were collected every 5 min. Fractions (1.5 ml) adjusted to pH 3.4 were frozen and then lyophilized for subsequent reconstitution and determination of CRF concentration. The functional viability of each assay was assessed at the end of each experiment by activating Na^+ channels with a 10-min pulse of 2 μM veratridine (Sigma).

2.4. Determination of CRF

CRF was measured by radioimmunoassay (Torres-Aleman et al., 1983). We used synthetic rat/human CRF 41 (Peninsula Laboratories, Belmont, CA, USA) as standard, and rat/human CRF antiserum raised in rabbit rC70 in a final dilution of 1:400000 (gift from Dr. W. Vale, Salk Institute, La Jolla, CA, USA). The ^{125}I rat/human CRF was purchased from NEN Research Products DUPONT (Boston, MA, USA). Separation of the antigen-antibody complex was achieved by means of a second antibody, goat antiserum to rabbit G-immunoglobulins (H + L-chains) (Jackson ImmunoResearch Laboratories, Baltimore, MD, USA) plus 14% polyethylene glycol. The intra-assay and inter-assay coefficients of variation were less than 13%.

2.5. Statistics

Secretion curve data are expressed as percentages of the basal secretion. Basal CRF secretion was calculated in each case as the mean of CRF secretion in the two fractions collected immediately before a pulse application. In the figures, the value for fraction 0 represents this CRF secretion value. The data were analyzed by one- or two-way analysis of variance (ANOVA). Net secretion was calculated as the area under the hormone secretion curve and expressed as percentage of the total basal secretion in the same period of time. These latter data were analyzed by Student's two-tailed unpaired t-test. All data are expressed as the means ± S.E.M.

3. Results

3.1. Effect of N^G-nitro-L-arginine on interleukin-1-induced CRF secretion

As shown in Fig. 1, left panel, N^G-nitro-L-arginine (1 mM) infusion prior (from 20 min before) and simultaneously with interleukin-1 markedly reduced CRF release by the cytokine ($P < 0.0002$). Analysis of the total net secretion (Fig. 1, right panel) shows that inhibition of NO synthesis with N^G-nitro-L-arginine treatment resulted in 75.4% inhibition of the stimulatory effect on CRF secretion induced by interleukin-1 ($P < 0.0001$). However, the basal CRF release was not significantly affected by the 20-min pulse of N^G-nitro-L-arginine (percentage of total net secretion versus basal secretion: fraction number (FN)-1 = 107.4 ± 4.1, FN-2 = 119.7 ± 12.1, FN-3 = 128.2 ± 8.3, FN-4 = 104.9 ± 11.4; $P = 0.9$, n.s.).

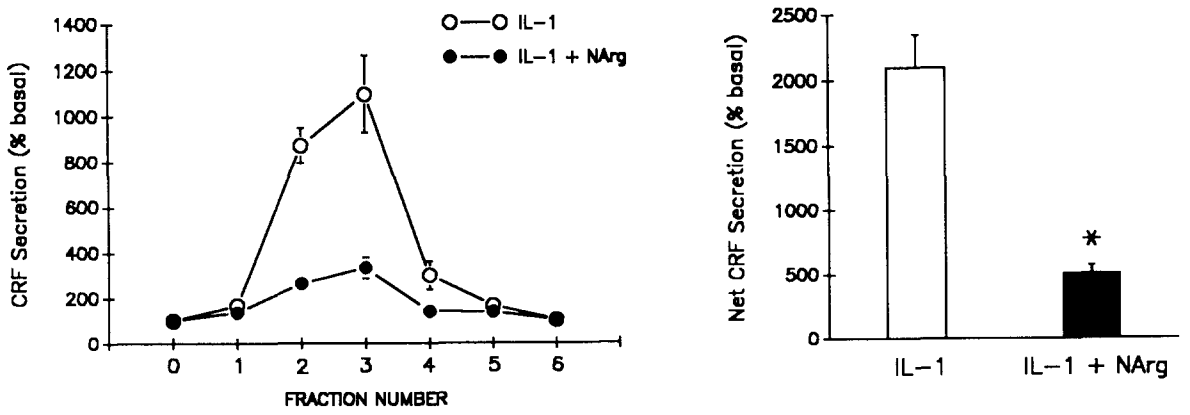

Fig. 1. Effect of N^G-nitro-L-arginine (1 mM) on CRF release in response to a 10-min pulse of interleukin-1β (3 pM) from five perifused hypothalami. Left panel shows the time course of CRF secretion in response to the different treatments. Fractions were collected every 5 min. Right panel represents percentage of total net CRF secretion versus basal secretion. Basal CRF secretion: interleukin-1 = 7.4 pg/ml, interleukin-1 + N^G-nitro-L-arginine = 8.8 pg/ml. Results are the means ± S.E.M. of six experiments.

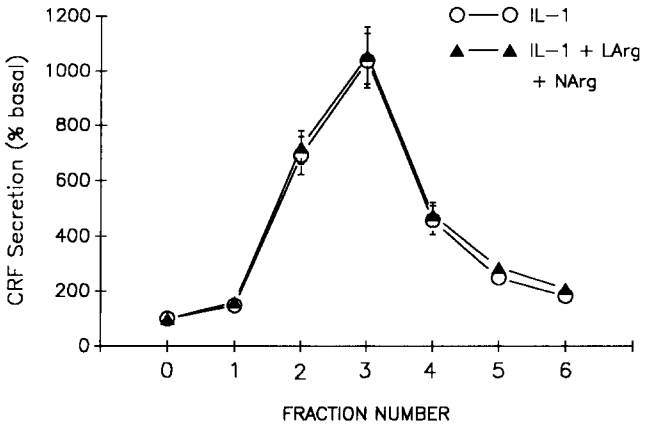

Fig. 2. Effect of L-arginine (1 mM) on CRF release from five hypothalami subjected to N^G-nitro-L-arginine (1 mM) and interleukin-1 (3 pM) treatment. Fractions were collected every 5 min. Basal CRF secretion: interleukin-1 = 6.8 pg/ml, IL1 + L-arginine + N^G-nitro-L-arginine = 6.4 pg/ml. Results are means ± S.E.M. of three experiments.

3.2. Effect of N^G-nitro-L-arginine plus L-arginine on interleukin-1-induced CRF secretion

If N^G-nitro-L-arginine were abolishing interleukin-1-induced CRF release by specifically inhibiting NO synthesis, incubation of hypothalami with the NO precursor L-arginine should prevent such an effect. Indeed, L-arginine (1 mM) pre- and co-infused with N^G-nitro-L-arginine (1 mM) plus interleukin-1 (3 pM) reversed the inhibitory action of N^G-nitro-L-arginine on interleukin-1 action. As can be seen in Fig. 2, when L-arginine + N^G-nitro-L-arginine were pre-infused the hypothalami showed a CRF response to interleukin-1 similar to their first response to interleukin-1 prior to the infusion of these substances ($P = 0.71$, n.s.). Basal CRF release was not significantly affected by the 20 min pulse of L-arginine (percentage of total net secretion versus basal secretion: FN-1 = 93.8 ± 10.5, FN-2 = 110.6 ± 7.1, FN-3 = 111.9 ± 12.0, FN-4 = 73.2 ± 23.3; $P = 0.36$, n.s.).

3.3. Effect of hemoglobin on interleukin-1-induced CRF secretion

To further ascertain whether NO is involved in the CRF-releasing activity of interleukin-1, we added hemoglobin (10 μm), which binds NO, to the perifusion medium prior to (from 20 min before) and simultaneously with interleukin-1 (3 pM). As shown in Fig. 3, inside panel, we first observed a reduction of basal, non-stimulated, CRF secretion by hemoglobin ($P < 0.0001$). Addition of interleukin-1 at min 20 of this 30-min hemoglobin pulse failed to stimulate CRF release and the effect differed significantly from the enhanced CRF secretory activity elicited by a subsequent pulse of interleukin-1 alone ($P < 0.05$).

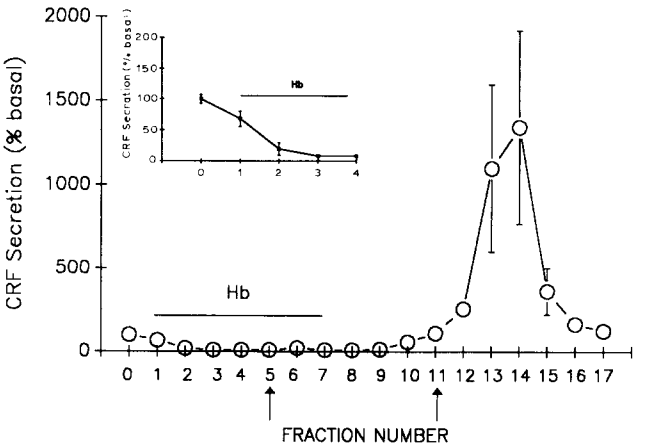

Fig. 3. Effects of two 10-min pulses of interleukin-1 (3 pM) on CRF release from hypothalami submitted to a pre- and co-incubation with hemoglobin (10 μM) and after clearance of hemoglobin from the perifusion medium. Arrows show the time points when interleukin-1 pulses started. Inside panel shows the effect of a 20-min HB pulse on basal CRF secretion. Fractions were collected every 5 min. Results are the means ± S.E.M. of four experiments.

3.4. Effect of indomethacin plus N^G-nitro-L-arginine on interleukin-1-induced CRF secretion

We have previously shown that the inhibitor of prostaglandin synthesis, indomethacin (14 μM), causes a reduction of 65% of the total net CRF secretion induced by interleukin-1 (3 pM) (Cambronero et al., 1992a). If NO and prostaglandins were mediating interleukin-1 effects on CRF secretion through independent pathways, then inhibition of both prostaglandin synthesis and NO synthase activity should result in an additive attenuation of interleukin-1-induced CRF release. However, as shown in Fig. 4, pretreatment and co-incubation with either N^G-nitro-L-arginine (1 mM) or N^G-nitro-L-arginine (1 mM) plus indomethacin (14 μM) pre- and co-incubation inhibited interleukin-1-stimu-

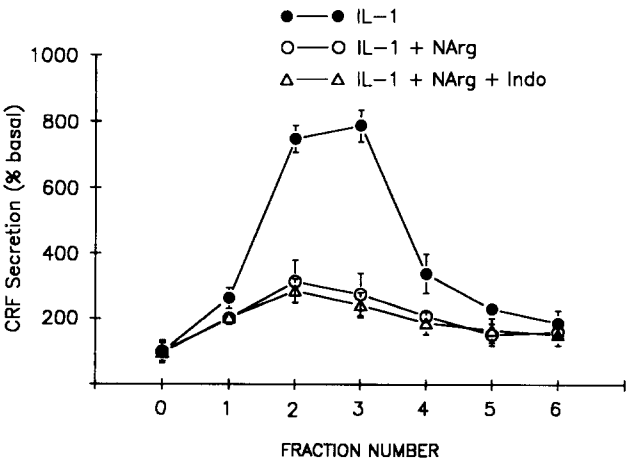

Fig. 4. Effects of preincubation with N^G-nitro-L-arginine (1 mM) or N^G-nitro-L-arginine (10 mM) plus indomethacin (14 μM) on CRF release from five hypothalami stimulated with interleukin-1 (3 pM). Fractions were collected every 5 min. Results are means ± S.E.M. of four experiments.

lated CRF secretion to the same degree ($P < 0.006$). Nevertheless, it should be noted that the reduction in CRF secretion induced by N^G-nitro-L-arginine alone (70.2%) might be too great to allow detection of further decreases due to a combination of the treatments. On the other hand, neither N^G-nitro-L-arginine nor N^G-nitro-L-arginine + indomethacin pretreatment affected basal CRF release ($P = 0.20$, n.s. and $P = 0.28$, n.s., respectively; data not shown).

4. Discussion

This work shows that, using an in vitro perifusion system, pretreatment of hypothalami with either N^G-nitro-L-arginine, an inhibitor of NO synthase, or the NO scavenger hemoglobin, results in a marked reduction of CRF release in response to interleukin-1, suggesting the involvement of NO in this interleukin-1 action. NO is produced from L-arginine by the enzyme NO synthase, and N^G-nitro-L-arginine – a L-arginine derivative – inhibits NO synthase by competing with L-arginine. Hence, the likelyhood of specificity of the effect reported here was supported by the complete blockade of N^G-nitro-L-arginine effect by the simultaneous addition of L-arginine to the perifusion medium. In agreement with results of previous studies (Brunetti et al., 1993; Costa et al., 1993; Karanth et al., 1993), basal CRF secretion was not altered by N^G-nitro-L-arginine. However, our results also showed that hemoglobin inhibited basal CRF secretion, a discrepancy which could be due to the different mechanism by which N^G-nitro-L-arginine and hemoglobin interfere with NO function (i.e., whereas N^G-nitro-L-arginine inhibits NO production, hemoglobin removes NO from the extracellular space).

The results of the present study consistently support a role of NO in the mediation of the interleukin-1 effect on CRF secretion. Either interfering with the synthesis of NO by inhibiting NO synthase with N^G-nitro-L-arginine (1 mM), or N^G-nitro-L-arginine methyl ester (unpublished observations), or inactivating NO with hemoglobin (10 μM), resulted in a marked inhibition of this hypothalamic secretory effect of the cytokine. These results are in agreement with a recent report (Brunetti et al., 1993) of the ability of N^G-nitro-L-arginine to block interleukin-1-induced CRF release from rat hypothalamic cell cultures. However, our results are in disagreement with a study (Costa et al., 1993) published during the progress of the present work, in which such an effect of interleukin-1 on CRF secretion was modified by neither the inhibitor of NO synthesis, N^G-monomethyl-L-arginine, nor by hemoglobin. Although such a disagreement is difficult to explain, it could be due to differences in particular aspects of the experimental procedures used (i.e., continuous perifusion system vs. static incubation, pretreatment with the inhibitor vs. direct co-incubation, etc.). In fact, divergent results with these procedures have also been found regarding the stimulation of hypothalamic CRF by another cytokine, interleukin-2 (Cambronero et al., 1992b; Karanth et al., 1993; Navarra et al., 1991). Also, we consider that a plausible explanation for the divergent results might be that, whereas we preincubated with the NO synthase inhibitor, Costa et al. (1993) added the inhibitor simultaneously with interleukin-1. Pretreatment with NO synthase inhibitors has previously proved to be necessary for these agents to be effective. Thus, in learning models in which NO has been implicated as a mediator of synaptic plasticity, inhibitors of NO synthesis have been shown to be amnestic if injected between 15 min (i.c. injection) and 30–60 min (i.p. injection) pretraining, but no closer to the training trial (Chapman et al., 1992; Hölscher and Rose, 1993). Similarly, experiements showing interference of NO synthase inhibitors with the induction of long-term potentiation (LTP) consistently involved preincubation of brain slices with the inhibitors for times varying from 15 min to 2.5 h before tetanization (Haley et al., 1992; Nowicky and Bindman, 1993; O'Dell et al., 1991; Williams et al., 1993). In addition, the inhibition of the excitatory glutamatergic agonist *N*-methyl-D-aspartate (NMDA)-induced cyclic GMP formation by inhibitors of NO synthesis was shown from studies involving 10–15 min preincubation of slices with these agents (East and Garthwaite, 1990, East and Garthwaite, 1991). Indeed, we observed a lack of effect of N^G-nitro-L-arginine in pilot studies in which hypothalami were concurrently incubated with interleukin-1 and N^G-nitro-L-arginine, without a pretreatment period with the inhibitor (unpublished observations). Recently, it was reported that the washout curve of NO after brain insufflation resulted in a half-life of 10.5 min (Meulemans, 1994). If such were the case, it is reasonable to hypothesize that, in order to interfere with the transduction mechanisms initiated by interleukin-1, the NO synthase inhibitor needs to have been present for a certain period before the cytokine reaches the hypothalamic tissue. In fact, the possibility remains that once this response has started, additional NO production could lead to negative modulation of interleukin-1 response, either through a direct action or by inducing other modulatory mechanisms. This model would fit with the results of Costa et al. (1993) showing a decrease of interleukin-1-induced CRF secretion by co-incubation of hypothalami with either the NO precursor, L-arginine, or the NO donors, molsidomine or sodium nitroprusside. The same model would apply for the in vivo results reported by Bluthé et al. (1992) and Rivier and Shen (1994) showing respectively that interleukin-1-induced behavioural and endocrine effects

(i.e., the release of the hypothalamic adenocorticotropin hormone, ACTH) were potentiated by the simultaneous administration of an inhibitor of NO synthesis.

Cytokines are known to induce synthesis of NO in different tissues. In particular, interleukin-1 has been shown to stimulate NO production in blood cells (Kosaka et al., 1992) and astrocytes (Vigne et al., 1993; Simmons and Murphy, 1993). Evidence is also appearing for a role of NO in neuropeptide and neurotransmitter release (Guevara-Guzman et al., 1994; Lutz-Bucher and Koch, 1994; Montague et al., 1994; Ota et al., 1993), including CRF (Karanth et al., 1993). Our results further support this view and strengthen the hypothesis of an involvement of NO in the mechanisms of CRF release by cytokines (Karanth et al., 1993). Thus, Karanth et al. (1993) have presented evidence that NO is involved in the activation of CRF release by interleukin-2, but not by noradrenaline. They hypothesized a chain of events involving several cells for the cytokine effect. According to the authors, interleukin-2 would stimulate acetylcholine release, which would subsequently activate NO synthase to produce NO in a different neuron. Then, NO would diffuse to CRF neurons, where it could activate the cyclooxygenase enzyme-stimulating prostaglandin production, which then would lead to cAMP-dependent CRF release.

We have also explored the possibility that the involvement of NO in interleukin-1 stimulation of CRF secretion could either be related to the transduction mechanism already established for this cytokine involving prostaglandin activation (Cambronero et al., 1992a; Navarra et al., 1991; Guaza and Borrell, unpublished observations), or represent different biochemical pathways which act either independently or synergistically on this interleukin-1 secretory action. Treatments inducing increased levels of hypothalamic NO result in elevated prostaglandin release (Rettori et al., 1992), and a similar activation of cyclooxygenase enzymes by NO has been reported in immune cells (Salvemini et al., 1993). Interestingly, in human fetal fibroblasts, NO donors were found to increase cyclooxygenase activity stimulated by interleukin-1 (Salvemini et al., 1993). NO is supposed to activate these enzymes by binding to their heme-containing group. In our study, the inhibition of interleukin-1-induced CRF release in the presence of N^G-nitro-L-arginine plus the cylooxygenase inhibitor, indomethacin, was similar to that obtained with N^G-nitro-L-arginine alone. Since we previously showed that the same dose of indomethacin attenuates this secretory effect of the cytokine (Cambronero et al., 1992a), the lack of an additive inhibitory effect of the NO and prostaglandin inhibitors suggests that they impair CRF release at different points along the same interleukin-1-induced chain of events. Otherwise, if NO and prostaglandin would involve independent mechanisms stimulated by interleukin-1 action in a parallel way, we should have expected a further inhibitory effect when their respective inhibitors were applied together instead of separately. However, it is difficult to establish from these results whether prostaglandin production depends upon NO activation, or vice versa. From the above studies we could speculate about the former possibility, i.e., that NO comes first and stimulates prostaglandin release. However, the converse possibility might also account for our results. In fact, a prostaglandin E_2 action at the hypothalamus (thermoregulatory modulation) appears to occur via activation of local NO synthase (Amir et al., 1991). Nevertheless, these results should be interpreted cautiously since the decrease induced by the NO synthase inhibitor (70.2%) could be too great to allow the detection of further decreases induced by a combination of the treatments. Further work is necessary to elucidate the sequence of involvement of NO and prostaglandins in the chain of biochemical events involved in the stimulatory effect of interleukin-1 on CRF release.

The mechanisms of action mediating interleukin-1 actions in the brain are of interest in view of the central effects induced by peripherally stimulated interleukin-1 and the limited accessibility of the brain for this large hydrophylic protein. Given its easy diffusibility, NO is a good candidate to act as a messenger since it might be activated by the cytokine in circumventricular organs and then diffuse to nearby hypothalamic regions. Moreover, small amounts of peripheral interleukin-1 appear to be able to reach the brain (Banks et al., 1989) and this cytokine may also be locally produced within the brain (Schultzberg, 1992), and NO could also act as a messenger at this level. This study provides evidence for a role of NO in the mechanisms mediating hypothalamic CRF secretion by interleukin-1 stimulation, supporting the hypothesis that CRF-releasing cytokines, as shown for interleukin-2 (Karanth et al., 1993), act via the NO pathway (McCann et al., 1994). It is also suggested that NO might interact with prostaglandin activation as connected steps in the same chain of transduction mechanisms involved in the hypothalamic action of interleukin-1 on CRF secretion.

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