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Effects of pyrogenic immunomodulators on the release of corticotrophin-releasing factor-41 and prostaglandin E_2 from the intact rat hypothalamus *in vitro*

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1 The actions of the following pyrogens: lipopolysaccharide (LPS), polyinosinic:polycytidylic acid (Poly-I:C), human interleukin (IL)-1α and IL-1β, human IL-6 and rat interferon (INF) on corticotrophin-releasing factor-41 (CRF-41) and prostanglandin E_2 (PGE_2) release from the intact rat hypothalamus *in vitro* have been studied.

2 Rat hypothalami were incubated *in vitro* in an artificial cerebrospinal fluid. Immunoreactive (ir)-CFR-41 and PGE_2 released into the medium were measured by two-site enzyme amplified immunometric assay (EAIA) and radioimmunoassay (RIA) respectively.

3 Human IL-6 (1 to 10,000 iu ml^{-1}) caused a dose-dependent release of irCRF-41, rising to a maximal 3–4 fold increase over basal at the highest dose tested. Human IL-1α (1 to 1000 iu ml^{-1}), human IL-1β (1 to 1000 iu ml^{-1}), poly-I:C (10 pg ml^{-1} to 100 μg ml^{-1}) and rat INF (1 to 10,000 IRu ml^{-1}) all failed to alter irCRF-41 release.

4 LPS (1 mg ml^{-1}) caused a 35% decrease in irCRF-41 release; however, over the dose-range of 0.1 μg ml^{-1} to 100 μg ml^{-1}, LPS failed to alter irCRF-41 release. The decreased irCRF-41 release in response to LPS (1 mg ml^{-1}) was accompanied by a decrease in the subsequent 56 mM KCl stimulation of irCRF-41.

5 Human IL-1α and IL-1β (1000 iu ml^{-1}) were able to stimulate the release of $irPGE_2$ from intact hypothalami, causing a 2 fold increase over basal release. Poly-I:C (100 μg ml^{-1}), LPS (0.1 μg ml^{-1} to 1 mg ml^{-1}), rat INF (10,000 IRu ml^{-1}) and human IL-6 (1 to 10,000 iu ml^{-1}) all failed to alter $irPGE_2$ release.

6 In conclusion, these results suggest that the *in vitro* release of CRF-41 and PGE_2, in response to pyrogens, are mediated via different cytokines. In view of this it is possible that different cytokines may mediate the temperature, prostaglandin and hypothalamo-pituitary-adrenocortical axis activation seen during pyrogenic stimulation *in vivo*.

Keywords: Prostaglandin; corticotrophin releasing factor; pyrogen

Introduction

A number of observations have led to the hypothesis that the fever inducing properties of both endogenous and exogenous pyrogens are mediated via the action of prostaglandins. Firstly, cyclo-oxygenase inhibitors prevent fever in response to pyrogens (Vane, 1971). Secondly, prostglandin E_2 (PGE_2) causes fever when injected into the third ventricle of cats and rabbits (Milton & Wendlandt, 1971). Thirdly, peripherally administered pyrogens cause an increase in both blood (Rotondo *et al.*, 1988) and cerebrospinal fluid (Feldberg & Gupta, 1973; Feldberg *et al.*, 1973. Harvey *et al.*, 1975) concentrations of prostaglandins. Furthermore, many pyrogens have been shown to activate the hypothalamo-pituitary-adrenal (HPA) axis (Yasuda & Greer, 1978; Woloski *et al.*, 1985; Uehara *et al.*, 1987; Tsagarakis *et al.*, 1989; Gwosdan *et al.*, 1990; Milton *et al.*, 1992b). For both interleukin-1 (IL-1) and polyinosinic:polycytidylic acid (Poly-I:C) such activation has been shown to occur via a corticotrophin-releasing factor-41 (CRF-41)-dependent mechanism (Uehara *et al.*, 1987; Milton *et al.*, 1992b). However, there is much controversy over the site of action of both the fever-inducing and HPA axis activating properties of pyrogens.

Some pyrogens demonstrate a distinct lag phase before inducing fever or HPA axis activation (Rotondo *et al.*, 1988; Milton *et al.*, 1992b). This argues either for a delay in reaching the sites of action or a requirement for the generation of other endogenous mediators. In the case of peripherally administered IL-1, the pyrogenic and adrenocorticotrophic hormone (ACTH) inducing properties may not be mediated via a direct action on the hypothalamus since radiolabelled IL-1 is unable to cross the blood brain barrier (Dinarello *et al.*, 1978). However, it is possible that certain pyrogenic substances may penetrate the blood brain barrier during fever or may by synthesised in the brain itself. Certainly there is evidence for the existence of central IL-1 and IL-6 neuronal networks and binding sites (Farrar *et al.*, 1987; Spangelo & MacLeod, 1990). Recent studies using *in vitro* methods have suggested that the endogenous pyrogens IL-1 and IL-6 stimulate the release of hypothalamic CRF-41 via a prostaglandin-dependent mechanism (Navarra *et al.*, 1991). The rises in blood levels of PGE_2, unlike those in $PGF_{2\alpha}$ mirror the increases in body temperature (Milton *et al.*, 1993) and PGE_2 is the most potent of the prostaglandins which activate body temperature increases (Milton & Wendlandt, 1971). In view of this we examined the effect of a number of endogenous and exogenous pyrogens on the secretion of immunoreactive (ir) CRF-41 and PGE_2 from the rat hypothalamus *in vitro*.

Methods

Rat hypothalamus in vitro

The incubation of rat hypothalami was performed *in vitro* by the well established technique of Bradbury *et al.* (1974).

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Adult male Wistar derived rats were decapitated, the skull cap removed and the exposed brain deflected backwards after the optic nerves had been cut. The hypothalamus was then dissected out and transferred to a tube containing 1 ml of artificial cerebrospinal fluid (composition mM: NaCl 126, KCl 6, Na_2HPO_4 1, $MgSO_4.7H_2O$ 0.877, $NaHCO_3$ 22, $CaCl_2$ 1.45, D-glucose 10 and 0.1% w/v BSA; pH 7.4) in a shaking water bath at 37°C with an atmosphere of 95% O_2:5% CO_2. After a 60 min pre-incubation period, during which the medium was changed three times, tissue was incubated for 20 min to establish basal release. Tissue was then incubated for a further 20 min in the presence of test substances. The basal and test incubations were then repeated and after a further 20 min incubation with basal medium alone, the tissue was exposed to a depolarizing stimulus with medium containing 56 mM KCl. The Na^+ concentration was reduced from 126 mM to 76 mM to maintain osmolarity. The release of irCRF-41 and $irPGE_2$ into the medium was measured after extraction and concentration of the samples on Sep-Pak C_{18} cartridges.

Test substances

LPS (from *E.coli* serotype 055:B5), Poly-I:C and rat interferon (2.7×10^5 IRu mg^{-1} protein) were obtained from Sigma Chemical Co, Poole, Dorset UK. Human IL-1α (100,000 iu μg^{-1} protein), human IL-1β (100,000 iu μg^{-1} protein) and human IL-6 (5,000 iu μg^{-1} protein) were obtained from the National Institute for Biological Standards and Controls, Mill Hill, UK.

Extraction of irCRF-41 and $irPGE_2$

(i) *irCRF-41* Sep-Pak C_{18} cartridges were pre-wetted with methanol followed by water. Acidified samples were applied, unbound material washed off with acetic acid, and irCRF-41 eluted with 2 ml acetonitrile (75%). Supernatants were dried down under a stream of nitrogen prior to assay. Recoveries for synthetic irCRF-41 added to samples were 86 ± 9%.
(ii) *$irPGE_2$* Sep-Pak C_{18} cartridges were similarly prepared and samples applied. Unbound material was washed off with 10 ml H_2O followed by 0.5 ml methanol. Bound PGE_2 was eluted with 1.5 ml methanol. Recoveries of PGE_2 added to extracts were 94 ± 5%.

Enzyme amplified immunometric assay for rat CRF-41

IrCRF-41 was measured with an ultra-sensitive, highly specific enzyme amplified immunometric assay (Milton *et al.*, 1990a) modified with a monoclonal antibody (KCHMB003) to rat CRF-41 (Milton *et al.*, 1990b). A commercial enzyme-amplified substrate system-AMPAK, (Dako Diagnostics, Cambridge, UK) was used to develop the colour.

NUNC 96 well Immunoplates were coated with 100 µl/well of rC70 antiserum (1:20,000) in carbonate buffer (pH 9.6) and incubated overnight at 4°C. The plates were washed to remove unbound antiserum and incubated with 250 µl/well of 5% lactose (w/v) and 0.2% casein (w/v) in carbonate buffer for 1 h at room temperature. Plates were then washed, air dried and sealed with acetate sealer before storage at 4°C in a desiccator.

Synthetic rat CRF-41 standard solutions (prepared by serial doubling dilutions in assay buffer (50 mM Tris 0.1% BSA; 0.1% Triton X-100; pH 7.4) to cover the range 0.4 to 200 pM) or samples were added to pre-coated plates (100 µl/well) and incubated overnight at 4°C. The plates were washed four times with washing buffer prior to addition of 100 µl/well anti-rat CRF-41 monoclonal antibody (KCHMB003)-alkaline phosphatase conjugate (1:1,000) and incubation for 2 h at room temperature. The plates were again washed four times with washing buffer, AMPAK substrate (100 µl/well) added and incubated for 15 min at room temperature. The AMPAK amplifier (100 µl/well) was then added. The plates were again incubated at room temperature and after sufficient colour had developed the reaction was stopped by addition of 0.3 M sulphuric acid (50 µl/well). The absorbance of each well at 495 nm was measured and irCRF-41 levels in samples calculated by interpolation from the standard curve.

The assay had a sensitivity of 1 fmol ml^{-1} and showed cross-reactivity with oxidised CRF-41 (79%). Serial dilutions of synthetic CRF-41, Sep-Pak C_{18} purified hypothalamic extracts and Sep-Park C_{18} extracts of medium from hypothalamic incubations all showed parallel curves.

Radioimmunossay of prostaglandin E_2

$IrPGE_2$ concentrations were determined by RIA with a kit purchased from Du Pont Inc. This assay had a sensitivity of 0.7 fmol ml^{-1} and showed cross-reactivity with PGE_1, (3.2%). Serial dilutions of standard PGE_2 and Sep-Pak C_{18} extracts of medium from hypothalamic incubations showed parallel curves.

Statistics

Results were calculated as means ± s.e.mean from at least five determinations and the degree of significance tested by one-way analysis of variance. For all studies a P value of <0.05 was considered significant.

Results

Basal irCRF-41 and $irPGE_2$ release

Considerable variation in the mean basal release was observed between different experiments. The mean (± s.e.mean) for irCRF-41 ranged from 3.0 ± 1.0 fmol/hypothalamus per 20 min ($n = 9$) to 12.3 ± 3.1 fmol/hypothalamus per 20 min ($n = 9$). The mean (± s.e.mean) for $irPGE_2$ ranged from 88 ± 20 fmol/hypothalamus per 20 min ($n = 6$) to 403 ± 66 fmol/hypothalamus per 20 min. Basal release in successive samples taken from the same group of hypothalami showed no statistically significant variation (Table 1). To account for within-group variations in irCRF-41 or $irPGE_2$ release, all data from each individual hypothalamus were expressed as a percentage of its own basal release during the preceding incubation period. Comparisons were made between mean % basal release from groups of at least 5 hypothalami treated with each test substance.

Effects of pyrogenic immunomodulators

ir CRF-41 release The addition of human IL-6 over a dose-range of 1 to 10,000 iu ml^{-1} caused a dose-dependent release of irCRF-41 from intact rat hypothalami incubated *in vitro* (Figure 1). The threshold dose was 100 iu ml^{-1}, with 1,000 iu ml^{-1} causing a 2–3 fold increase and 10,000 iu ml^{-1} causing a 3–4 fold increase, relative to the basal release. Basal release after the first stimulation with human IL-6 was not significantly different from the first basal and the tissue was able to respond to a subsequent dose of IL-6 with a comparable release of irCRF-41.

The addition of LPS over a dose range of 0.1 µg ml^{-1} to 100 µg ml^{-1} failed to alter irCRF-41 release from intact rat hypothalami incubated *in vitro*, relative to the basal release (Figure 2). Addition of a depolarized stimulus of 56 mM KCl to tissues treated with LPS resulted in a 2 fold increase in irCRF-41 release. The release of irCRF-41 in response to 56 mM KCl did not differ between groups, given different doses of LPS. Basal release after the first stimulation with LPS was not affected and irCRF-41 release was not altered during a subsequent incubation with LPS.

However, LPS in a dose of 1 mg ml^{-1} caused a 35%

Table 1 Variability in immunoreactive corticotrophin releasing factor-41 (irCRF-41) and ir prostaglandin E_2 (irPGE$_2$) release from the rat hypothalamus *in vitro*

Incubation Period (20 min)	*irCRF-41* (fmol/hypothalamus per 20 min)	*irPGE$_2$* (fmol/hypothalamus per 20 min)
1	8.1 ± 1.4	99 ± 20
2	7.5 ± 1.0	117 ± 31
3	7.9 ± 1.4	105 ± 28
4	7.0 ± 0.9	88 ± 20

Results are shown as mean (± s.e.mean) irCRF-41 or irPGE$_2$ release from at least five observations per group.

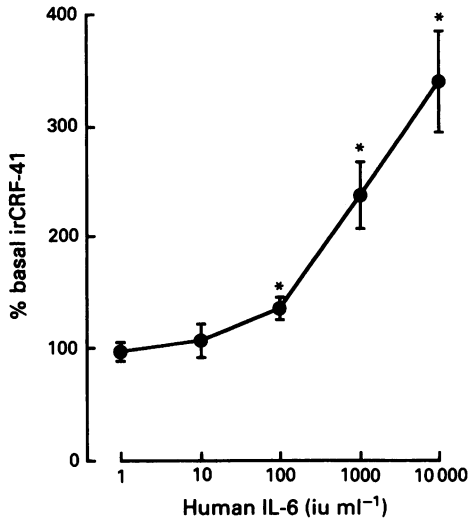

Figure 1 Effect of human interleukin-6 (human IL-6) on the release of immunoreactive corticotrophin releasing factor-41 (irCRF-41) from the rat hypothalamus *in vitro*. Results expressed as % basal release, are shown as mean ± s.e.mean from at least five observations. *$P<0.05$ by one way analysis of variance compared to unstimulated controls.

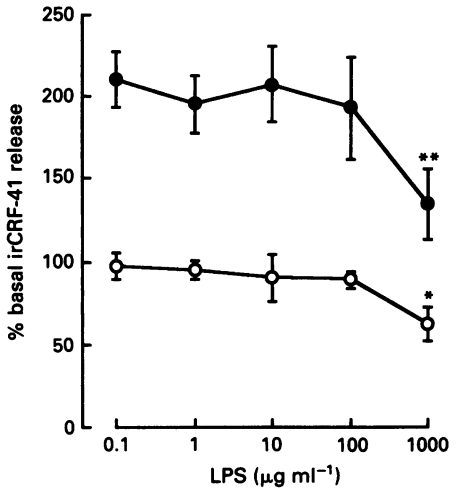

Figure 2 Effect of lipopolysaccharide (LPS) (*E.coli* 0.55:B5) on release of immunoreactive corticotrophin releasing factor-41 (irCRF-41) from the rat hypothalamus *in vitro* (○) and on the 56 mM KCl stimulated release of irCRF-41 (●). Hypothalami were stimulated with LPS before challenge with 56 mM KCl. Results, expressed as % basal release, are shown as mean ± s.e.mean from at least five observations. *$P<0.05$ compared with unstimulated controls; **$P<0.05$ compared with controls treated with media alone followed by 56 mM KCl by one-way analysis of variance.

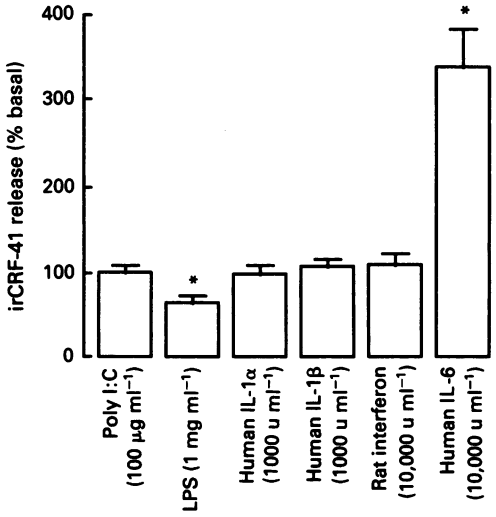

Figure 3 Effect of pyrogenic immunomodulators (for abbreviations see text) on the release of immunoreactive corticotrophin releasing factor-41 (irCRF-41) from the rat hypothalamus *in vitro*. Results, expressed as % basal release, are shown as mean ± s.e.mean from at least five observations. *$P<0.05$ by one way analysis of variance compared to unstimulated controls.

decrease in irCRF-41 release which was observed in both the first and second incubation periods. The release of irCRF-41 was non-significantly decreased in the intervening basal period. The 56 mM KCl stimulation evoked a 2 fold increase in irCRF-41 relative to basal release in control groups, which was reduced by 30% in hypothalami preincubated with 1 mg ml^{-1} LPS (Figure 2).

The addition of poly-I:C (10 pg ml^{-1} to 100 μg ml^{-1}), human IL-1α (10 to 1,000 iu ml^{-1}), human IL-1β (10 to 1,000 iu ml^{-1}) or rat interferon (INF) (1 to 10,000 IRu ml^{-1}) failed to alter irCRF-41 release from intact rat hypothalami incubated *in vitro*, relative to the basal release (Figure 3). Addition of a depolarizing stimulus of 56 mM KCl to tissues treated with these pyrogens resulted in a 2–4 fold increase in irCRF-41 release. The release of irCRF-41 in response to 56 mM KCl did not differ between groups given different doses of these pyrogens. Basal release after the first stimulation with pyrogen was not affected and neither was the irCRF-41 release during a subsequent incubation with pyrogen.

When tissues were incubated with acetylcholine (10 nM) a 2.5 fold increase in the release of irCRF-41 was observed. This response was not affected by the presence of poly-I:C (1 μg ml^{-1}) in the incubation medium (Table 2).

irPGE$_2$ release Poly-I:C (1 μg ml^{-1}), LPS (0.1 μg ml^{-1} to 1 mg ml^{-1}), rat INF (10,000 IRu ml^{-1}) and human IL-6 (1 to 10,000 iu ml^{-1}) all failed to alter irPGE$_2$ release from intact rat hypothalami incubated *in vitro*, relative to the basal release (Figure 4). Basal release after the first stimulation with pyrogen was unaffected and irPGE$_2$ release was not altered during a subsequent incubation with any of these pyrogens.

Both human IL-1α and IL-1β at a dose of 1,000 iu ml^{-1} caused a 2 fold increase in irPGE$_2$ release from intact rat hypothalami incubated *in vitro*, relative to the basal release (Figure 4). Basal release after the first stimulation with IL-1α or IL-1β was not significantly different from the first basal

Table 2 Effect of acetylcholine and polyinosinic: polycytidilic acid (poly-I:C) on immunoreactive corticotrophin releasing factor-41 (irCRF-41) from the rat hypothalamus *in vitro*

	irCRF-41 (% basal)
Poly-I:C (1 $\mu g\ ml^{-1}$)	107 ± 15
Acetylcholine (10^{-9} M)	237 ± 30*
Poly-I:C + acetylcholine	252 ± 41*

Results, expressed as % basal release, are shown as mean ± s.e.mean from at least five observations. *$P<0.05$ by one way analysis of variance compared to unstimulated controls.

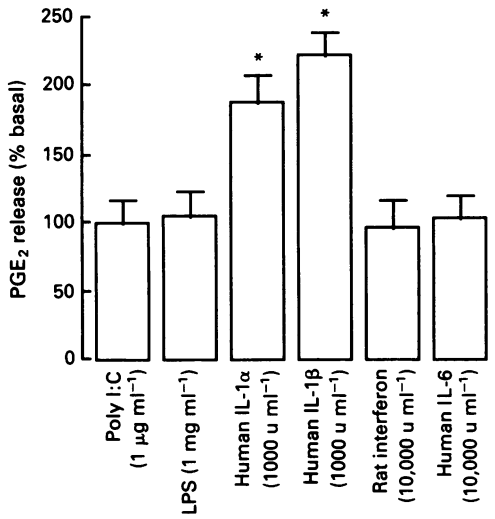

Figure 4 Effect of pyrogenic immunomodulators (for abbreviations, see text) on the release of immunoreactive prostaglandin E_2 ($irPGE_2$) from the rat hypothalamus *in vitro*. Results expressed as % basal release, are shown as mean ± s.e.mean from at least five observations. *$P<0.05$ by one way analysis of variance compared to unstimulated controls.

samples and $irPGE_2$ release was similarly elevated during a subsequent incubation with IL-1α or IL-1β.

Discussion

The rat hypothalamus *in vitro* technique has previously been well validated (Bradbury *et al.*, 1974; Hillhouse 1975; Buckingham & Hodges 1977; Hillhouse & Milton 1989a,b) for the study of regulators of CRF bioactivity and immunoreactivity. The experimental design was such that all tissue samples, subjected to incubations with test substances, were exposed to a depolarizing 56 mM KCl stimulus at the end of the experiment. This test confirmed the viability of each tissue and enabled any adverse or prolonged effects of test substances on irCRF-41 release to be detected. Previous studies have confirmed that the hypothalamus incubated in this manner remains viable for up to 3 h (Hillhouse, 1975) and none of the experimental procedures involved incubations of longer duration. The variability in the basal release of irCRF-41 and $irPGE_2$ between groups may reflect differences in experimental animal groups or procedures. However, within group variation in basal release was sufficiently small to allow comparison of consecutive samples. The tissues were taken at the same time (between 09 h 00 min and 10 h 00 min) to eliminate contributions from hormonal circadian rhythms. The variability in the basal release, however, prevented comparison of actual irCRF41 levels stimulated by test substances in different experiments. The problems of variability in basal release were overcome by expression of irCRF-41 or $irPGE_2$ release from each individual hypothalamus as a percentage of its own basal release during the preceding incubation period, so making meaningful comparisons possible. However, since the underlying causes of the differences in basal release may also influence the responsiveness of the tissue to a range of secretagogues only qualitative comparisons between individual experiments can be made.

The hypothalamus has been shown to mediate the stimulatory effect of LPS on ACTH secretion in rats (Yasuda & Greer 1978) and poly-I:C activates the HPA axis in rabbits via a CRF-41 dependent mechanism (Milton *et al.*, 1992b). In this study neither of these exogenous pyrogens stimulated the secretion of either irCRF-41 or $irPGE_2$ from the rat hypothalamus *in vitro*. Indeed at the highest dose tested (1 $mg\ ml^{-1}$) LPS caused a significant inhibition of irCRF-41 release which was long lasting and probably reflects a non-specific toxic effect. This strongly suggests that, at least in rats, neither of these exogenous pyrogens activate CRF-41 containing neurones via a direct effect at the hypothalamic level.

The failure of poly-I:C either to stimulate or to potentiate irCRF-41 secretion from the rat hypothalamus is interesting in view of our recent observations that peripheral CRF-41 plays a role in the febrile and prostaglandin responses to poly-I:C in rabbits (Milton *et al.*, 1992a). In these studies there was a lag phase of 30–60 min before an increase in circulating ir-cortisol was observed in response to poly-I:C (Milton *et al.*, 1992b). This suggests either a delay in poly-I:C reaching its site of action or the contribution of another endogenous activator of the HPA axis. Two such potential mediators are interferon and IL-1, both of which are activated by poly-I:C (Stringfellow, 1984; Souvannavong & Adam, 1990). In rats, injection of human γ-INF into the third cerebral ventricle, stimulates ACTH secretion (Gonzalez *et al.*, 1990) and IL-1 activates the HPA axis via a CRF-41-dependent mechanism (Uehara *et al.*, 1987). However, in the present study neither of these compounds had any effect on CRF-41 secretion from the rat hypothalamus *in vitro*. The rat interferon preparation used was chosen because it was obtained from poly-I:C stimulated cells and because of the suggested species specificity for some of the actions of this cytokine. Our results suggest that in the rat the main site of action of IL-1 and interferon is not the hypothalamic CRF-41 neurones.

The failure of IL-1 to stimulate CRF-41 release from the intact hypothalamus is in direct contrast to the observations of other groups using a similar preparation (Loxley *et al.*, 1992; Hagan *et al.*, 1992). However, effects due to endogenous glucocorticoids, which act to suppress hypothalamic CRF-41 release in response to cytokines (Loxley *et al.*, 1992) may account for these observed differences. The observations of an increase in the hypothalamic tissue content of CRF-41 in response to IL-1 (Hagan *et al.*, 1992) could be due to the presence of amino acids in the incubation medium and the observed release may well be a result of such synthesis rather than CRF-41 release in direct response to IL-1. The results from this study also contrast with previous reports of IL-1 stimulation of CRF-41 release in bisected rat hypothalamic fragments *in vitro* (Tsagarakis *et al.*, 1989; Navarra *et al.*, 1991). In view of the differences in experimental design and the nature of the neurones exposed by such dissection, methodological differences may provide an explanation for the many contradictory results. The different assay techniques may also play a role in the observed results. In our study, we used an ultrasensitive immunometric assay which is both more sensitive and specific for rat CRF-41 than available radioimmunoassays (Milton *et al.*, 1990a). The basal release of irCRF-41 reported for the rat hypothalamic

fragment system corresponded to 8.6 ± 0.8 fmol/hypothalamus per 20 min (Tsagarakis *et al.*, 1988) and is within the range found in the present study. However, the release in response to a 56 mM KCl stimulation was greater, giving a ten fold increase over basal compared to the 2–4 fold increase observed in the present study. These results suggest that the dissection of the hypothalamus may expose more CRF-41 producing neurones to the test substance. The comparison of parallel incubations rather than serial samples from the same tissue may also be a source of differences in the results obtained.

A recent study using cyclo-oxygenase inhibitors has suggested that the IL-1 and IL-6 stimulated release of ir CRF-41 from rat hypothamic fragments is prostaglandin-dependent (Spangelo & MacLeod, 1990). The molecular weight of IL-1 (17,000) is sufficiently large to suggest that it is unlikely for the material to diffuse far into the hypothalamic blocks studied *in vitro*. The neurones stimulated are likely, therefore, to be close to the surface of the tissue. The majority of the CRF-41 perikarya are contained within the paraventricular nucleus (Sawchenko & Swanson, 1985; Palkovits *et al.*, 1985; Petrusz *et al.*, 1985) which is situated near the surface of the hypothalamic blocks used in this study (Bradbury *et al.*, 1974). In the hypothalamic fragments method, other hypothalamic neurones may also be exposed to test substances. The IL-1 activation of prostaglandins has previously been reported for rabbit hypothalamic fragments and provides the basis for a bioassay for IL-1 (Symans *et al.*, 1987). In this study we showed the ability of IL-1 to stimulate PGE_2 release from the hypothalamus *in vitro* without any concomitant increase in CRF-41 release. This argues against a PGE_2-mediated action of IL-1 on the hypothalamic CRF-41 neurones. The ability of intravenously administered IL-1 to activate the HPA axis and irCRF-41 release into the hypophyseal portal circulation *in vivo* (Sapolsky *et al.*, 1987) suggests that such activation is at the hypothalamic level. However, the failure of indomethacin implants to attenuate the IL-1 induced ACTH rises suggests that such *in vivo* activation is not prostaglandin-dependent (Sapolsky *et al.*, 1987). However, Rivier & Vale (1991) have suggested that IL-1β activation of ACTH may have a prostaglandin-dependent component. These observations are consistent with the view that the pyrogenic and ACTH-inducing actions of IL-1 are mediated via different mechanisms. The failure of IL-1 to cross the blood-brain barrier (Dinarello *et al.*, 1978; Coceani *et al.*, 1988) has led to the suggestion that peripherally generated IL-1 gains access to the brain via the circumventricular organs (Coceani *et al.*, 1988; Katsuura *et al.*, 1990). One such site is the organum vasculosum lamina terminalis which is thought to be the site whereby IL-1 activates hypothalamic CRF-41 containing neurones via a prostaglandin-dependent mechanism (Coceani *et al.*, 1988).

Another cytokine which is secreted by activated immune cells during the acute phase of an immune response is IL-6 (Spangelo & MacLeod, 1990). Like IL-1 it causes both fever and activation of the HPA axis. Both *in vitro* (Navarra *et al.*, 1991) and *in vivo* (Naitoh *et al.*, 1988) experiments suggest that such activation is mediated via the production of CRF-41. Our results confirm that IL-6 stimulates the secretion of CRF-41 from the rat hypothalamus *in vitro*. However, this was not accompanied by a concomitant release of PGE_2 suggesting that this prostaglandin is not involved in the activation of CRF-41 release by IL-6. This is in agreement with the results of Bernadini *et al.* (1989) who reported that $PGF_{2\alpha}$, but not PGE_2, can stimulate the release of CRF-41 from rat hypothalamic explants. Further evidence in favour of prostaglandins being involved in the IL-6 activation of CRF-41 secretion has been provided from experiments using inhibitors of prostaglandin synthesis in hypothalamic fragments (Navarra *et al.*, 1991).

In conclusion, our results suggest that in the rat: (i) the exogenous pyrogens, LPS and Poly-I:C, and the endogenous pyrogens, IL-1 and INF, do not activate the HPA axis via a direct action at the level of the hypothalamic CRF-41 containing neurones; (ii) the endogenous pyrogen, IL-6, can activate hypothalamic CRF-41 neurones via a PGE_2-independent mechanism; (iii) IL-1 stimulates PGE_2 secretion from the hypothalamus without any concomitant change in CRF-41 secretion.

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