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CENTRAL SINGLE AND CHRONIC ADMINISTRATION OF MORPHINE STIMULATES CORTICOSTERONE AND INTERLEUKIN (IL)-6 IN ADJUVANT-INDUCED ARTHRITIS

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Adjuvant-induced arthritis (AA) in the rat is a chronic inflammatory stress in which circulating corticosterone and interleukin (IL)-6 levels are elevated. In addition, there are profound neuroendocrine changes associated with the development of hind-paw inflammation which have major implications for the ability of the rat to respond to stress. Central injection of morphine is able to increase plasma corticosterone and circulating IL-6 concentration in control animals. In present study we have determined the effects of a single and repeated injection of morphine into the lateral ventricle of control and AA animals on plasma corticosterone, circulating IL-6 levels and course of hind-paw inflammation in AA rats. In the present study we found a sustained increase in plasma corticosterone both after single and repeated injection of morphine in control and AA rats and an increase of the level of circulating IL-6 in AA rats after repeated injection of morphine. These data suggest that alternative systems distinct from Athose activated in response to acute stress are activated by morphine in the AA animals. The similarity with the sustained increase in corticosterone following LPS injection suggest that central opiates may be involved in mediating HPA axis and cytokines response to inflammatory stress.

Key words: *morphine, corticosterone, Interleukin-6, adjuvant arthritis*

INTRODUCTION

There is a growing interest in the connections between the central nervous system and immune response. Activation of the hypothalamo-pituitary-adrenal (HPA) axis in response to an acute stressors or acute immune challenge is characterised by an increase in ACTH release from the anterior pituitary which stimulates corticosterone release from the adrenal cortex. Pro-opiomelanocortin (POMC) mRNA is increased in the anterior pituitary and within the hypothalamus corticotrophin-releasing factor (CRF) and arginine-vasopressin (AVP) mRNAs are increased in the parvocellular cells of paraventricular nucleus (PVN) (1).

Adjuvant-induced arthritis (AA) is a chronic, immunologically mediated disease which is associated with chronic activation of the HPA axis (2—4). In contrast to normal animals, rats with AA are unable to mount a significant response to acute stressors such as ip. hypertonic saline, restraint or noise (1, 2). There have been previously demonstrated that despite the chronic activation of pituitary-adrenal axis seen in AA, CRF mRNA in the PVN and CRF peptide release into the hypophysial portal blood are paradoxically decreased (5). However, AVP mRNA in the parvocellular cells of the PVN and AVP peptide release into the hypophysial portal system are increased suggesting that AVP takes over as the major stimulator of the axis (3, 5—7).

In contrast to the lack of response seen with acute physical and psychological stressors, the corticosterone response to acute immune stimulation using lipopolysaccharide (LPS) remains intact (5, 8). These data suggest alternative signalling mechanisms associated with acute stress and acute immune stimuli.

In addition to these neuroendocrine changes, AA is associated with activation of cell-mediated immunity, resulting in increased secretion of inflammatory cytokines, such as Interleukin (IL)-1 β , Tumour Necrosis Factor α (TNF α) (2, 3) and IL-6 (2). IL-6 exerts multiple action on the growth, differentiation and function of lymphoid and non-lymphoid cells and regulates various aspects of the immune response and its production during injury or infection (4). Plasma levels of IL-6 appear to mirror the changes in inflammation (9). IL-6 is an important immune mediator co-ordinating the activity of different immune cells with an important role in acute phase response (3). While IL-6 is generally considered to be a pro-inflammatory cytokine, Mihara and colleagues noted that administration of IL-6 was able to suppress the development of AA suggesting a protective role (5, 10).

Plasma concentration of IL-6 can be elevated by acute stress (6), and acute administration of IL-1 or endotoxin (7, 11). Injection of IL-1 or LPS into the lateral ventricle will increase plasma IL-6 (11—13) an effect which can be blocked by prior icv injection of IL-1 receptor antagonist suggesting a central mechanism.

Opiates such morphine have been reported to increase IL-6 through a centrally mediated, receptor dependent mechanism (11, 12, 14). The source of the increase in circulating IL-6 in response to these acute stimuli remains to be determined.

In present study we have administered morphine centrally in control and AA rats. The purpose of this study was first, to determine the effects of centrally given morphine in single and chronic administration on activation of the HPA axis by measuring plasma corticosterone. Secondly, we have investigated alterations in IL-6 concentration and course of the disease after repeated administration of morphine in adjuvant-arthritis.

MATERIALS AND METHODS

160 adult male PVG rats (Bantin & Kingman, UK; 7–8 weeks of age) divided into 4 groups (CT/SAL, CT/MF, AA/SAL, AA/MF-40 rats each group) were kept under heat and humidity controlled conditions in a 12 h light: 12 h dark cycle (lights on at 07.00 h). Animals were allowed laboratory chow and tap water ad libitum.

AA was induced by a single intradermal injection of 0.1 ml of a 10 mg/ml suspension of ground, heat-killed *Mycobacterium butyricum* in paraffin oil administered under light halothane anaesthesia. Control animals were injected with vehicle alone. In this model hind-paw inflammation is usually apparent 12–13 days after induction, clearly visible by day 14 and reached peak severity at day 21 (1). Ten days after adjuvant injection a guide cannula was inserted into the right lateral ventricle and a cannula inserted into the jugular vein for the removal of blood samples. Following development of hind-paw inflammation a pre-injection blood sample (0.5 ml) was removed.

During the first part of experiment, saline or morphine (10 µg in 4 µl) was infused into the lateral ventricle over 1 minute. A further blood sample (0.5 ml) for corticosterone estimation was withdrawn after 2 hours.

In the second part of experiment we have measured circulating IL-6 concentration after repeated infusion (into the lateral ventricle) of morphine (at days 13th, 14th and 15th) and rats hind-paws were taken for arthritic estimation by measuring the volume of arthritic and control paws.

Trunk blood was taken for plasma corticosterone measurements, in triplicate, by radioimmunoassay using antiserum supplied by Dr G. Makara (Institute of Experimental Medicine, Budapest, Hungary). The tracer was ¹²⁵I-corticosterone (INC Biomedicals, CA, USA) with a specific activity of 2–3 mCi/µg. The sensitivity of the assay was 25 ng/ml. The intraassay coefficient of variation was less than 12%. The IL-6 concentration in serum was measured in duplicate by ELISA method using commercially available kits (R&D System). The sensitivity of assay was 31 pg/ml.

The hind paw volume was measured in duplicate by the plethysmometr.

Statistical comparisons were made using Fisher PLSD test following one-way ANOVA. A $p < 0.05$ was considered significant.

RESULTS

Adjuvant-injected animals which did not exhibit hind-paw inflammation were excluded from the experiment. Results concerning acute MF administration and reporting the stimulating effect of MF on corticosterone secretion in CT and AA animals has already been published (15).

As reported previously, basal corticosterone levels were significantly ($p < 0.05$) elevated in AA rats with hind-paw inflammation (*Fig. 1*) compared with non-arthritic controls. In the control rats two hours following morphine injection (10 µg in 4 µl icv), there was a significant ($p < 0.01$) increase in plasma corticosterone compared with the pre-injection levels. Morphine was also able to evoke a highly significant ($p < 0.001$) increase in circulating corticosterone in AA animals. These levels were also significantly elevated ($p < 0.001$) over levels in morphine-treated control rats.

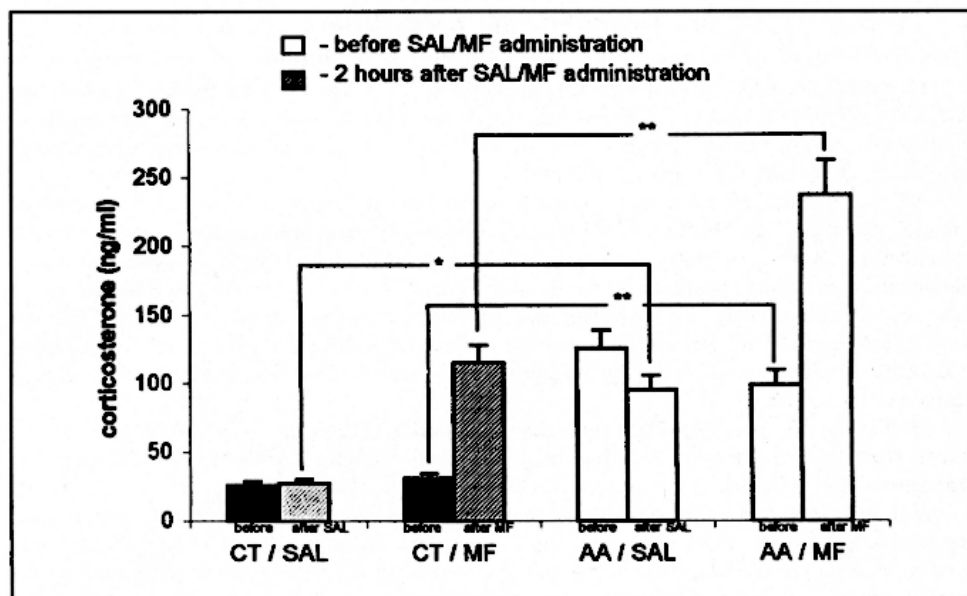


Fig. 1. Plasma corticosterone levels before (basal) and 2 hours following morphine ($10 \mu\text{g}$ in $4 \mu\text{l}$ 0.9% NaCl, icv) administration. Values represent mean \pm SEM. ** $p < 0.001$ CT/SAL vs CT/MF and AA/SAL vs AA/MF.

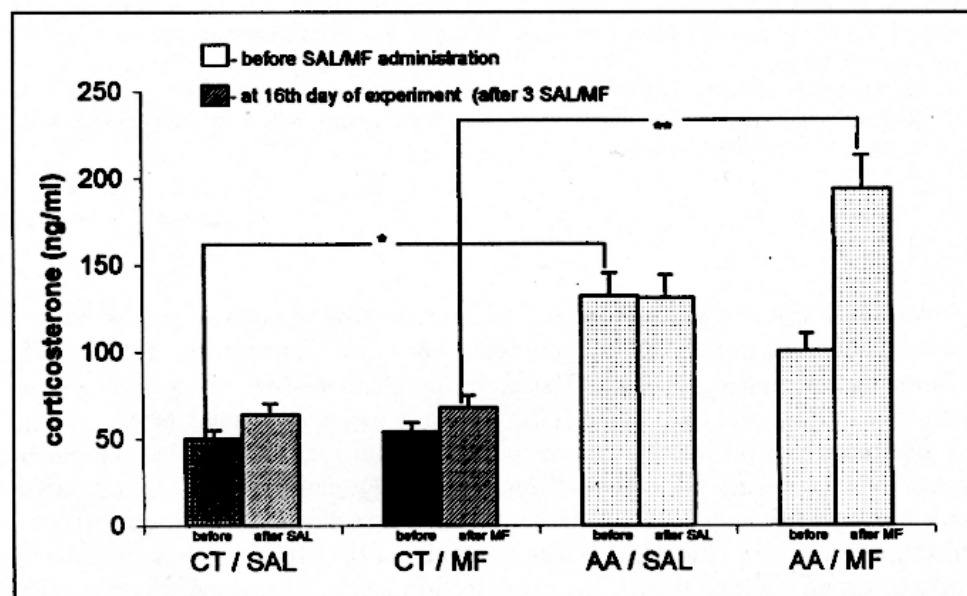


Fig. 2. Plasma corticosterone levels before and at day 16th following three subsequent morphine ($10 \mu\text{g}$ MF in $4 \mu\text{l}$ 0.9% NaCl i.c.v.) administration. Values represent mean \pm SEM. * $p < 0.01$; ** $p < 0.001$.

In second part of experiment, the onset level of corticosterone in AA rats (16th day of experiment) was significantly higher in comparison to CT group ($p < 0.01$) either in SAL or MF animals (*Fig. 2*). The i.c.v. administration of MF at days 13th, 14th and 15th caused significant increase of corticosterone concentration ($p < 0.001$) either in CT or AA animals.

IL-6 concentration in serum of CT animals were undetectable (*Fig. 3*). Repeated injections of morphine caused significant increase IL-6 concentration in serum of AA/MF animals in comparison to group AA/SAL ($p < 0.001$) (*Fig. 3*). The hind-paws volume at the onset of experiment in AA group was significantly higher in comparison to CT group ($p < 0.01$).

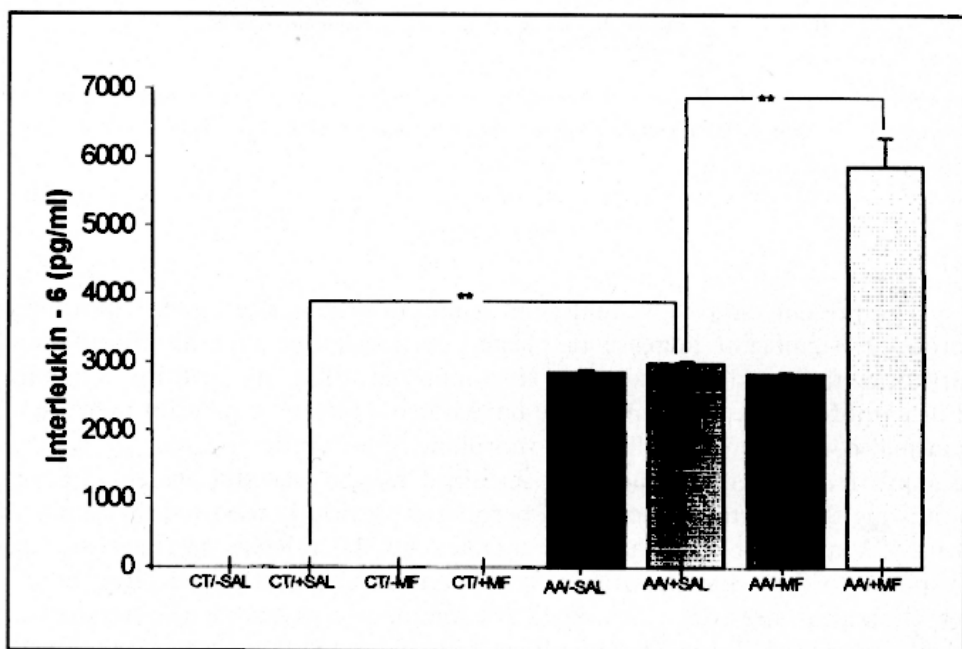


Fig. 3. Plasma IL-6 concentration in described groups before and after subsequent MF administrations (10 μ g MF in 4 μ l 0.9% NaCL i.c.v) at 31th, 14th and 15th day of experiment. Values represent mean \pm SEM. ** $p < 0.001$.

Repeated injections of morphine caused significant reduction of hind-paws volume in AA rats in comparison to AA group received SAL ($p < 0.01$) (*Fig. 4*).

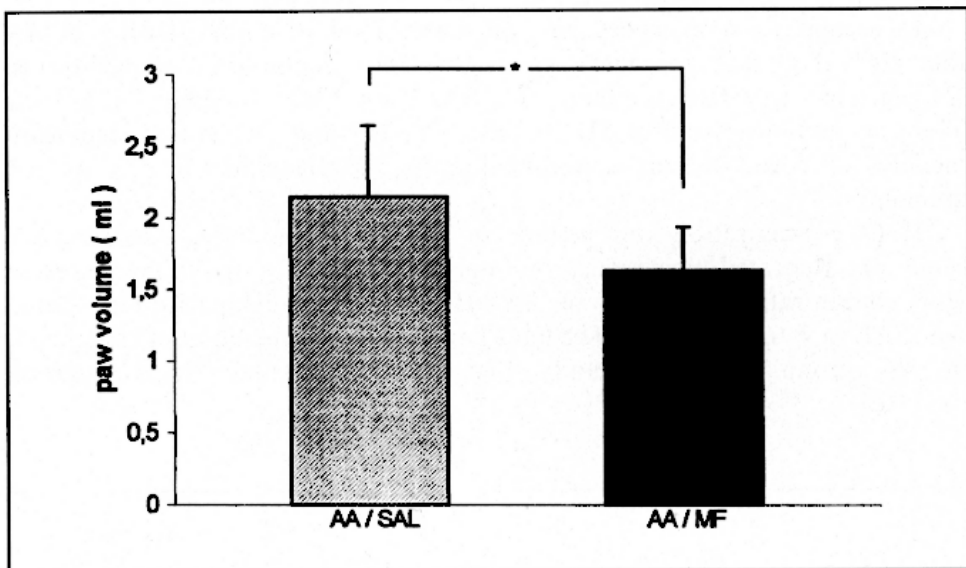


Fig 4. Paw volume. Values represent mean \pm SEM. * $p < 0.01$.

DISCUSSION

The present data show that central injection of opiate agonist morphine produces significant increase in plasma corticosterone in both control and arthritic rats and increase of IL-6 concentration in arthritic animals. Furthermore, in the AA animals we have noted a further significant increase in plasma corticosterone following morphine injection compared to control animals receiving morphine. This sustained release was still evident 2 hours following the injection. There have been have previously reported that animals with AA are unable to mount a significant CRF mRNA or corticosterone response to the acute stressors of ip. hypertonic saline (a mixed physical and psychological stressor), restraint (a predominantly psychological stressor) or noise stress (3, 5, 7, 15). These findings have suggested that associated with the development of the chronic inflammatory stress of AA there is an inhibition of the control mechanisms normally activated in response to stress. A similar situation is also found in patients with rheumatoid arthritis who, unlike non-arthritic patients, are unable to mount a cortisol response to the stress of surgery (16). However, it is not that the system is refractory to stimulation as we have been able to demonstrate a significant corticosterone response to acute injection of the immune modulator LPS (5). These data suggest a selective adaptation of HPA axis where, in response to an acute stressors, there is no further activation of the HPA axis. However, in response to an acute immune

stimulus, which in absence of a suitable glucocorticoid response might prove life threatening (17); this inhibition can be over-ridden or an alternative pathway activated to elicit the response. The sustained release of plasma corticosterone demonstrated in response to morphine in the present study is similar to the sustained release noted to LPS or indeed if endogenous opiates mediate the activation of the HPA axis evoked by acute immune activation.

In response to morphine repeated injection in the AA rat we have noted a significant increase in IL-6 concentration in serum. From these data it is clear that acute stimuli are able to exert actions on cytokine in tissues of the HPA axis. The same stimulus is able to exert IL-6 production.

These observations suggest multiple control mechanisms underlying these changes in different tissues.

Centrally administered morphine has previously been shown to reduce acute paw oedema following carageenin injection (18). In AA repeated subcutaneous injections of morphine, but not infusion of morphine, has been reported to attenuate inflammation (19). These effects were antagonized by naloxone suggesting a receptor mediated mechanism. Our own findings extend these previous observations to suggest that a possible mechanisms underlying the reported anti-inflammatory effects of central morphine may be due to an activation of HPA axis resulting in the release of anti-inflammatory glucocorticoids. In contrast to these findings, one group has reported a proinflammatory effect of morphine in AA following subcutaneous infusion (20). This discrepancy is intriguing, as is the observation that centrally-injected, high dose naloxone is also anti-inflammatory (18).

A possible explanation of these data concerns the mode of administration of morphine. The central or peripheral injection of a bolus of morphine may have been sufficient to reach the threshold limit, activate the HPA axis and hence exert a potent anti-inflammatory effect. In contrast, a slow infusion of the same amount of morphine may not have reached the necessary threshold. The reported proinflammatory actions of morphine are intriguing and may reflect the previously reported dose-dependent, biphasic effects of the opiates on immune function .

A possible mechanisms involved in mediating the anti-inflammatory effects of centrally injected morphine concerns IL-6. Opiates such morphine, β -endorphin and etorphin have been shown to increase circulating concentration of IL-6 through a specific receptor-mediated mechanism (14). One might speculate that the enhanced glucocorticoid release seen in the AA rats injected with morphine may be due to a further increase in the release of IL-6 above levels seen in AA (21, 22). Further studies will be required to test this hypothesis.

Further studies are required as well, to check if there is a role of IFN γ inhibition, or IL-10 or TGF β stimulation to play in reduction the degree of

inflammation, in response to morphine administration. As Ianaro (23) reported oral administration of TGF β (or induction of TGF β production by pro-inflammatory cytokines such as IL-2) significantly inhibits the course of inflammation. Moreover, Chao C *et al.* (24) described the possibility that TGF β could be an endogenous mediator of the immunosuppressive activity of morphine or endogenous opioids. Additionally Chernajovsky (25) described that TGF β was effective in lowering inflammation of joints with already established arthritis and inhibits the spread of the disease to other joints.

Additionally, Joosten *et al.* (26) observed that IL-10 elevated in murine collagen-induced arthritis slightly suppressed the arthritis. This suppression can go also by down-regulation tissue IL-1, TNF β and IFN γ gene expression suggesting that IL-10 or TGF β treatment could be an effective approach in arthritis. These observations are fulfilled with Scott M and Carr J (27) study describing significant decrease in IFN γ concentration (which belongs to pro-inflammatory cytokines) in the supernatants of splenocytes obtained from subchronic morphine-treated mice after antigen stimulation suggesting that morphine acts to suppress IFN γ at the transcriptional level.

In summary, we have noted changes in IL-6 concentration in serum of AA rats treated with morphine. These changes may be involved in the sustained elevation in plasma corticosterone seen in these animals. The similarities between our observation of the sustained increase in plasma corticosterone following central morphine injection, and those reported following acute injection on immune modulators such as IL-1 or LPS, suggest that similar mechanisms may be activated. The role of central opiate activation in mediating acute immune activation of HPA axis and the role of endogenous opiates in the chronic immune stimulated model of AA requires further experimentation.

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