Br. J. Pharmacol. (1993), 109, 289-290

SPECIAL REPORT

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Peripheral endotoxin induces hypothalamic immunoreactive interleukin-1β in the rat

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Interleukin-1 (IL-1) is a polypeptide produced by a variety of cells and contributes to the general host response to inflammation. It displays a wide spectrum of inflammatory, metabolic, physiological, haematopoietic and immunological activities. Brain cells, including neurones, microglia, endothelial cells and astrocytes can all produce IL-1β in response to various physiological and pathological stimuli. In this report we show that peripherally administered endotoxin stimulates the appearance of immunoreactive IL-1β (IL-1β) in the rat hypothalamus as measured by an ultrasensitive, highly specific enzyme amplified immunometric assay for rat IL-1β.

Keywords: Endotoxin; interleukin-1β; hypothalamus

Introduction The mechanism whereby exogenous pyrogens activate thermoregulatory mechanisms remains uncertain, although a number of molecular signals are thought to be involved including the cytokines and prostaglandins (Kluger, 1991). In patients with infectious disease in which endotoxin plays a role it is likely that several cytokines, including interleukin-1 (IL-1), contribute to the clinical picture. Many of the central nervous system responses to endotoxin such as fever, sleep and anorexia can be reproduced by either peripheral or central administration of IL-1β (Rothwell, 1991). These observations strengthen the hypothesis that peripherally generated cytokines mediate both the central and peripheral metabolic responses to endotoxin.

Central to this hypothesis, is the integrity of the blood brain barrier. This appears to be impermeable to IL-1 (Dinarello *et al.*, 1978), although the barrier may alter during sepsis or other pathological states. Alternatively, IL-1 may penetrate the brain at areas outside the barrier, such as the organum vasculosum lamina terminalis (Katsuura *et al.*, 1990). Another possibility is that IL-1 might be generated centrally and would not therefore need to cross the blood brain barrier. Such a central IL-1 network has been identified within the hypothalamus (Koenig, 1991). Furthermore, IL-1 has been shown to activate neuroendocrine cells via a direct action at the hypothalamus (Scarborough *et al.*, 1989). In an effort to characterize further the effect of endotoxin on neuroendocrine mechanisms we sought to investigate whether peripherally administered endotoxin might stimulate the production of hypothalamic IL-1β.

Methods *Animals* Male Wistar-derived rats, weighing 250-300 g, were injected intraperitoneally with either endotoxin (1st International Reference Preparation 57/7, NIBSC) or vehicle (sterile, endotoxin-free 0.9% saline) at 09 h 00 min. The animals were killed by decapitation at various time intervals following the injection and each hypothalamus was rapidly removed and immediately homogenized in 1 ml of RPMI tissue culture medium (ICN Flow). The homogenates were stored at − 70°C before assay.

IL-1β immunoassay IrIL-1β was measured with an ultrasensitive highly specific, enzyme amplified, immunometric assay for rat IL-1β using a sheep polyclonal antiserum (Bristow *et al.*, 1989). The capture antibody was DEAE-purified and the detection antibody immunoaffinity-purified on a rat IL-1β affigel 10 column. The detection antibody was then conjugated to alkaline phosphate with a kit (Pierce) and purified by h.p.l.c. to obtain a conjugate with high sensitivity and low non-specific binding characteristics. Nunc immunoplates were coated with the capture antibody (1 μg/well) in carbonate buffer at 4°C overnight. Following blocking of unbound sites with 5% lactose and 0.2% casein, recombinant rat IL-1β standards (Dr S. Poole, NIBSC, Potters Bar, Herts) in RPMI, or tissue extracts were diluted in assay buffer (50 mM Tris + 0.1% bovine serum albumin) and added to the plates (10 μl/well). Following overnight incubation at 4°C the conjugated detection antibody (1:1000) was added and incubated at room temperature for 2 h. After washing AMPAK (Novo Biolabs) substrate (100 μl/well) was added followed, after 15 min, by AMPAK amplifier (100 μl/well). The absorbance of each well at 492 nm was measured with a Titertek Multiskan MCC plate reader.

Results *IL-1β immunoassay* The immunoassay for rat IL-1β had a sensitivity of 3 pg ml^{-1} and showed cross-reactivity with both murine IL-1β (17.5%) and human IL-1β (13%). No cross-reactivity was observed with human IL-1α, human IL-6, human/rat corticotrophin-releasing factor-41 (CRF-41), arginine vasopressin and α-melanocyte stimulating hormone. The recovery of rat IL-1β added to the hypothalamic homogenate was 67%. IL-1β extracted from endotoxin stimulated

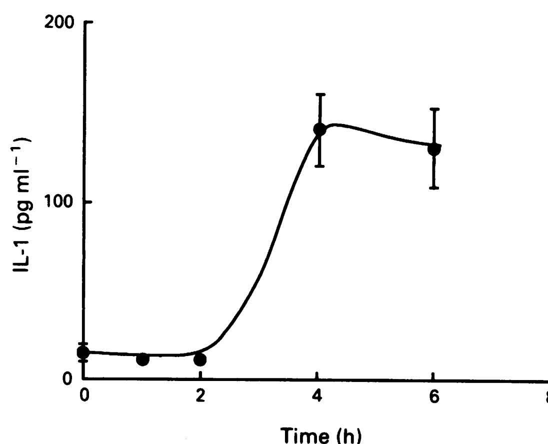

Figure 1 Time course of the effect of intraperitoneal endotoxin (250 μg/animal) on the immunoreactive interleukin-1β (IL-1) content of the rat hypothalamus. Each point represents the mean ± s.e.mean of at least four observations.

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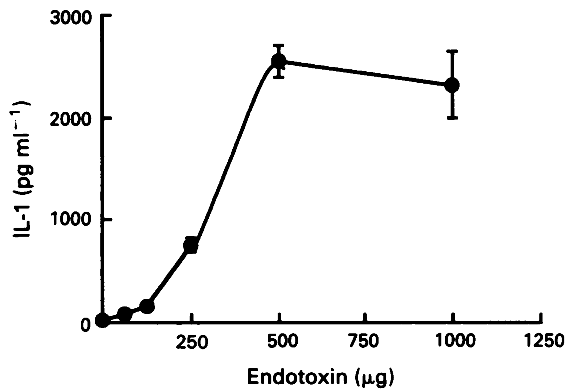

Figure 2 The effect of various doses of intraperitoneal endotoxin on the immunoreactive interleukin-1β (IL-1) content of the rat hypothalamus. Each point represents the mean ± s.e.mean of at least four observations.

rat hypothalami diluted in parallel with the standard curve (data not shown).

Time course When animals were treated with endotoxin (250 μg/animal) there was a delay of 2 h before any increase in the hypothalamic immunoreactive IL-1β content (Figure 1). This effect was maximal between 4–6 h. Control animals showed no response to vehicle control (data not shown).

Dose-dependancy The effect of endotoxin on hypothalamic IL-1β immunoreactivity was dose-dependent over the range of 125–500 μg (Figure 2). Control animals showed no response to vehicle control (data not shown).

Discussion This study presents some new insights into the mechanisms whereby endotoxin might activate central neuroendocrine systems. The cellular sources of brain IL-1β remain controversial. It appears to be present within neurones paricularly in areas involved in the regulation of anterior pituitary function (Koenig, 1991). Furthermore, microglia, brain endothelial cells and astrocytes have all been shown to produce IL-1β in response to various physiological and pathological stimuli (Benveniste, 1992). The IL-1β produced under these circumstances has been implicated in both tissue damage and repair. Our study indicates that endotoxin induces the synthesis of IL-1β in the brain either directly or via some secondary neural or humoral mediator. Of these possibilities the latter seems the most likely and it is possible that peripheral IL-1β itself might be one of these mediators. Previous reports have shown endotoxin-induced synthesis of IL-1β by use of immunocytochemistry and *in situ* hybridization (Van Dam *et al*., 1992; Ban *et al*., 1992) but this is the first report showing time- and concentration-dependance. In this short study we cannot completely exclude the possibility that the IL-1β found in the hypothalamus is transported from the peripheral circulation. This seems unlikely, however, in view of the fact that circulating concentrations of IL-1β are low and transport into the brain is less than 1% of circulating protein. In conclusion, endotoxin induces hypothalamic IL-1β and this may participate in the integration of the neuroendocrine responses to sepsis.

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(Received January 11, 1993
Accepted February 23, 1993)