Inactivation of the Cerebral NF κ B Pathway Inhibits Interleukin-1 β -Induced Sickness Behavior and c-Fos Expression in Various Brain Nuclei

Agnès Nadjar¹, Rose-Marie Bluthé¹, Michael J May², Robert Dantzer¹ and Patricia Parnet^{*,1}

¹UMR INRA 1244-CNRS-Université Victor Segalen Bordeaux II, Institut François Magendie, rue Léo Saignat, Bordeaux, Cedex, France;

²Department of Animal Biology, University of Pennsylvania, Philadelphia, PA, USA

The behavioral effects of peripherally administered interleukin-1 β (IL-1 β) are mediated by the production of cytokines and other proinflammatory mediators at the level of the blood–brain interface and by activation of neural pathway. To assess whether this action is mediated by NF κ B activation, rats were injected into the lateral ventricle of the brain with a specific inhibitor of NF κ B activation, the NEMO Binding Domain (NBD) peptide that has been shown previously to abolish completely IL-1 β -induced NF κ B activation and Cox-2 synthesis in the brain microvasculature. NF κ B pathway inactivation significantly blocked the behavioral effects of intraperitoneally administered IL-1 β in the form of social withdrawal and decreased food intake, and dramatically reduced IL-1 β -induced c-Fos expression in various brain regions as paraventricular nucleus, supraoptic nucleus, and lateral part of the central amygdala. These findings strongly support the hypothesis that IL-1 β -induced NF κ B activation at the blood–brain interface is a crucial step in the transmission of immune signals from the periphery to the brain that underlies further events responsible of sickness behavior.

Neuropsychopharmacology (2005) **30**, 1492–1499, advance online publication, 18 May 2005; doi:10.1038/sj.npp.1300755

Keywords: interleukin-1 β ; sickness behavior; NF κ B; c-Fos; *in vivo*

INTRODUCTION

When an organism becomes sick during the course of an infection, several changes occur, which are mediated by the central nervous system (CNS). These changes include regulated increase in body temperature, increase in slow wave sleep, activation of the hypothalamo–pituitary–adrenocortical (HPA) axis, decrease in locomotor activity, feeding, drinking and social interactions, and alterations in brain neurotransmitters. The physiological and behavioral components of sickness represent a highly organized strategy of the organism to fight infection referred to as ‘sickness behavior’ (Dantzer, 2001).

There has been a recent surge of interest in the behavioral effects of cytokines in neuropsychopharmacology. Several reasons account for that, including the fact that cytokine-induced sickness behavior is not the result of weakness and physical debilitation but appears to be the expression of a previously unrecognized motivational state that is triggered

by peripheral immune stimuli and reorganizes the organism’s priorities (Dantzer, 2004). In addition, the possibility of an intersection between sickness behavior and depression (Dantzer *et al*, 1999) has raised new and important issues in psychopathology.

Interleukin-1 β (IL-1 β), produced by activated macrophages and monocytes in contact with invading microorganisms, is an important cytokine for the induction of sickness behavior (Dantzer, 2001; Dinarello, 2000; Gabay and Kushner, 1999; Krueger *et al*, 1999; Mulla and Buckingham, 1999; Turrin and Plata-Salaman, 2000). Peripheral and central administration of IL-1 β induces all the central components of the acute phase reaction, and inhibition of its action by central administration of the IL-1 receptor antagonist (IL-1ra) attenuates IL-1 β -induced sickness behavior (Dantzer, 2001). However, in spite of its profound effects on the CNS, IL-1 β cannot passively cross the blood–brain barrier (BBB) formed by brain vascular cells because of its relatively high molecular weight and hydrophilic profile.

The brain action of IL-1 β is mediated by the formation of an heterodimeric complex associating the type I IL-1 receptor (IL-1RI) and the IL-1 receptor accessory protein. Using *in situ* hybridization, IL-1RI was found to be mainly expressed in brain barrier-related structures like the meninges, the choroid plexus, and vascular endothelium of the whole parenchyma (including the circumventricular

*Correspondence: Dr P Parnet, UMR INRA 1244-CNRS, Institut François Magendie, 146, rue Léo Saignat, 33077 Bordeaux Cedex, France, Tel: +33 5 57 57 37 05, Fax: +33 5 56 98 90 29, E-mail: patricia.parnet@bordeaux.inserm.fr

Received 22 October 2004; revised 2 March 2005; accepted 22 March 2005

Online publication: 31 March 2005 at <http://www.acnp.org/citations/Npp033105040497/default.pdf>

organs, or CVOs, that lack a BBB) (Ericsson *et al*, 1995). *In vitro* studies demonstrated that activation of IL-1 receptors led to the recruitment and phosphorylation of the receptor-associated kinases (IRAK1 and IRAK2) via the docking molecule MyD88. The subsequent phosphorylation of I κ B α , the inhibitor of NF κ B, results in the release of NF κ B that translocates to the nucleus. The DNA binding nuclear form of the transcription factor NF κ B is usually a heterodimer which typically includes one 50 kDa (p50) and one 65 kDa (p65) polypeptide (Baeuerle and Henkel, 1994; Moynagh *et al*, 1994; Hayden and Ghosh, 2004). It binds to its consensus sequence on target genes to promote transcription of a variety of genes like cytokines receptors, cytokines, prostaglandins, chemokines, and a few neuromediators (Miyamoto and Verma, 1995).

Immunohistochemical studies of p65-NF κ B in the brain of rats treated with IL-1 β injected intraperitoneally showed that activation of NF κ B was restricted to specific cell populations in the CVOs, the BBB, and at the interface between the cerebrospinal fluid (CSF) and the brain parenchyma (Nadjar *et al*, 2003). This distribution pattern was abolished in IL-1RI-deficient mice, confirming the crucial role of IL-1RI expressed on BBB cells and in CVOs for p65-NF κ B translocation in response to peripheral IL-1 β (Nadjar *et al*, 2003).

The general scheme of NF κ B pathway activation is well described: binding of IL-1 β to IL-1RI is followed by a cascade of phosphorylation that results in NF κ B nuclear translocation. This depends on the regulatory protein NEMO (NF κ B essential modifier) associated with a complex containing two kinases IKK α and IKK β (O'Neill and Greene, 1998). Once within the nucleus, NF κ B binds to its consensus sequence on target genes that promotes transcription of a variety of genes. The present study was designed to assess the functional consequences of NF κ B activation at the interface between the blood and the brain. For this purpose, a specific inhibitor of NF κ B activation was used. This inhibitor targets the (NEMO)-binding domain (NBD) of IKK α or IKK β specifically. Therefore, it inhibits NF κ B activation without inhibiting basal NF κ B activity (May *et al*, 2000). This inhibitor blocks TNF- α -induced NF κ B activation *in vitro* and attenuates peripheral and central responses to inflammation *in vivo* (May *et al*, 2000; Dasgupta *et al*, 2004). To target NF κ B activation at the blood-brain interface level, this peptide was injected into the lateral ventricle of the brain, and we checked its capacity to diffuse from there to its potential sites of action and significantly attenuate IL-1 β -induced translocation of NF κ B (Nadjar *et al*, in press).

In the present report, we show that inactivation of NF κ B at the blood-brain interface inhibits both the cerebral activation response to peripheral IL-1 β , as measured by expression of the early activated protein c-Fos (Sagar *et al*, 1995) and the behavioral alterations induced by intraperitoneally administered IL-1 β .

MATERIALS AND METHODS

Subjects

Male Wistar Crl: (WI) IGS BR rats (125–150 g) were obtained from Charles River, Brussels, Belgium. After

arrival at the facility, they were housed in groups of five in transparent polycarbonate cages (42.6 $\times$ 27.8 $\times$ 18.8 cm) and acclimated to the laboratory for at least 2 weeks. They were maintained under standard colony conditions in a temperature (23 $\pm$ 1°C), humidity (40%) controlled room and on a 12–12 h light/dark cycle. Food (U.A.R., Epinay-sur-Orge, France) and water were available *ad libitum*. For social exploration tests, juveniles male rats (28–35 days of age) of the same strain served as social stimuli and were housed in transparent polycarbonate cages (42.6 $\times$ 27.8 $\times$ 18.8 cm) in groups of 10 in a different room. The investigators adhered to the guidelines of the Institute for Laboratory Animal Research (ILAR) published in *Guide for Care and Use of Laboratory Animals*. Every effort was made to minimize animal numbers and suffering in the experiments.

Surgical Procedures and Treatments

For central injections, a stainless-steel guide cannula (23-gauge, 7 mm length) was implanted unilaterally 1 mm above the lateral ventricle. For this surgery, rats were anesthetized intraperitoneally with a mixture of ketamine (Imalgene 1000, Rhône Mérieux, Lyon, France) and xylazine (Bayer Pharma, Puteaux, France)—at doses of 61 mg and 9 mg/kg, respectively—and secured in a Kopf stereotaxic instrument (Tujunga, CA). Coordinates were with toothbars 5 mm above the interaural zero, 0.6 mm posterior to the bregma, 1.5 mm lateral, and 3.2 mm below the skull surface, at the point of entry (Swanson, 1998). At this point, the rat body weight was 160–200 g. Rats were allowed a 2-week recovery period before behavioral testing. On the test day, treatment substances were administered by gravity into the lateral ventricle using a 30-gauge needle, over a 30-s period, in freely moving rats. Following the injection, social exploration, activity, food intake, body weight, and fever were measured at different intervals.

Treatments

Recombinant rat IL-1 β (rrIL-1 β , biological activity: 317 IU/mg, NIBSC, Potters Bar, UK) was dissolved in 0.9% saline whereas NBD peptide was dissolved in dimethylsulfoxide (DMSO) (Sigma-Aldrich Corporation, St Louis, MI). The dose of rrIL-1 β (60 μ g/kg, i.p.) was selected from a previous dose–effect study (Anforth *et al*, 1998). The dose of NBD peptide (36 μ g, intracerebroventricular (i.c.v.)) was selected on the basis of previous *in vivo* data (May *et al*, 2000) and experiments demonstrating its ability to block cerebral rrIL-1 β -dependent Cox-2 induction (Nadjar *et al*, in press). NBD peptide was dissolved in a solvent represented by non-diluted DMSO. This solvent did not induce any behavioral modifications (data not shown) as previously demonstrated (Bluthe *et al*, 1997). NBD peptide (36 μ g) or its excipient was injected i.c.v. as a pretreatment 1 h before i.p. injection of rrIL-1 β (60 μ g/kg) or saline. All substances were administered in a volume of 2 μ l (i.c.v.) or 600 μ l (i.p.).

For the clarity of presentation of results, the treatments schedule is notified on each figure and in experimental procedure.

Experimental Procedure

NBD peptide and rrIL-1 β effects on duration of social exploration and immobility. Animals were kept under reversed light–dark conditions (lights on at 2100 h, lights off at 0900 h). Behavioral observations were carried out during the dark phase of the light–dark cycle, between 0900 and 1700 h, using a video camera under red light illumination. Rats were isolated (single in a cage) 24 h before the experiment in transparent polycarbonate cages (42.6 $\times$ 27.8 $\times$ 18.8 cm), and they were habituated to experimental procedures by testing them two times daily for three successive days before the experiment. Sickness behavior induced by rrIL-1 β was assessed by: (1) decreases in duration of the social exploration of a conspecific juvenile introduced into the home cage of the test animal; (2) increases in duration of immobility of the test animal during the observation session. The procedure used to monitor social exploration had been previously validated (Bluthe *et al*, 1999). At 1 day before the experiment, baseline social exploration was assessed. The time spent by the experimental rat in social exploration consisted of ano-genital sniffing, body sniffing and grooming of the juvenile. It was measured during a 4-min period by a trained observer sitting in a different room and using a computerized program. The day after, rats were first injected i.c.v. with DMSO or NBD peptide then, 1 h after, i.p. with saline or rrIL-1 β , between 0900 and 1030 h, then tested with different juveniles on repetitions of the behavioral test to sustain a high level of social exploration. Behavior (duration of social exploration and immobility) was monitored at 0, 2, 4, 6, and 24 h after saline or rrIL-1 β treatments (Kent *et al*, 1992). Each rat received only one treatment combination. Saline-treated rats always remained active, which is why immobility data for these animals are not represented in Figure 1.

NBD peptide and rrIL-1 β effects on body weight and food intake. At the end of each behavioral session (at time 0, 2, 4, 6, and 24 h), the resident rat and the food tray were weighed on a top-loading balance accurate to 0.01 g. The weight of food was cumulated over a period of 24 h.

Tissue Preparation

For c-Fos expression analysis, rats were first injected i.c.v. with DMSO or NBD peptide and 1 h later i.p. with saline or rrIL-1 β , between 0900 and 1030 h. At 1 h after treatment, animals were injected with a lethal dose of pentobarbital. Brains were fixed by intracardiac perfusion of 4% paraformaldehyde (PF) in 0.1 M NaH₂PO₄/Na₂HPO₄ buffer, pH 7.5 (phosphate buffer, PB). Brains were removed and postfixed in the same fixative solution overnight at 4°C and cryoprotected in 30% sucrose in 0.1 M PB. Coronal 30 μ m cryostat sections were kept in anti-freeze solution (30% ethylene glycol, 30% glycerol, 25% Tris buffer (TB) sterile, and 15% sterile water) at –20°C until processing for immunohistochemistry.

Immunodetection of c-Fos

After washing-off of the cryoprotectant, free-floating sections were incubated for 1 h in TBS 1 $\times$ containing 1% BSA and 0.3% Triton X-100. Sections were then incubated

overnight at room temperature with a rabbit polyclonal antiserum raised against c-Fos (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1/10 000 in TBS 1 $\times$, 1% BSA, 0.3% Triton X-100. Tissue sections were rinsed in TBS 1X, treated 30 min with TBS 1 $\times$ containing 1% of H₂O₂, rinsed three times and incubated for 2 h with biotinylated donkey antiserum recognizing rabbit IgG (Amersham Pharmacia Biotech Europe, Freiburg, Germany) diluted 1/2000 in TBS 1 $\times$, 1% BSA, 0.3% Triton X-100. After three rinses, avidin–biotin peroxidase diluted 1/1000 in TBS 1 $\times$ (Vector laboratories, Burlingame, CA) was added for 2 h at room temperature. Then, the peroxidase reaction product was developed using diaminobenzidine and the nickel-enhanced glucose oxidase method (Shu *et al*, 1988), thus giving a black precipitate concentrated in the nucleus of the cells.

Microscopy

Brain sections were examined under a light microscope (Nikon Eclipse E 400) and the images were captured by a high-resolution digital Nikon DXM 1200 camera (Nikon Corporation, Champigny-sur-Marne, France). Camera aperture, magnification, light power, and exposure time were fixed for all images. ACT-1 software (Nikon Corporation, Champigny-sur-Marne, France)-generated images were stored on a personal computer. Image editing software (Adobe Photoshop, Adobe Systems, San Jose, CA) was used to adjust size, brightness, and contrast for photographs.

Quantification of c-Fos immunoreactive cells was performed with the aid of the public domain NIH-imaging software (Scion, Frederick, MD) coupled to a high-resolution digital Nikon DXM 1200 camera (Nikon Corporation, Champigny-sur-Marne, France). Images were thresholded to produce a binary image and c-Fos-immunoreactive nuclei were counted using the “Analyze particles” function. This procedure was very straightforward given the existence of c-Fos-immunoreactive cells as distinct dense black nuclei on a very light background. For all brain areas analyzed, counts were taken from all the consecutive sections showing this structure, across both hemispheres and these counts were averaged to produce a mean.

Statistical Analysis

All data are expressed as means $\pm$ SEM.

Duration of social exploration (s), body weight change (g), and food intake (g) were analyzed by a two-way ANOVA with treatment as a between-subject factor and time (2, 4, 6, 24 h) as a within-subject factors. *Post hoc* comparisons of individual group means were carried out by Fisher’s test. Since immobility was never observed in saline-treated groups, the duration of immobility in saline group was not represented and a two-way ANOVA (treatment $\times$ time) was used to compare the DMSO/rrIL-1 β group to the NBD peptide/rrIL-1 β group.

Data from immunohistochemistry experiments were analyzed by a two-way ANOVA (pretreatment and treatment). *Post hoc* comparisons of individual groups means were carried out using the Newman–Keuls test. Data were transformed when equality of variance or normality test failed. In all cases, a level of $p < 0.05$ was considered as statistically significant.

RESULTS

NBD Peptide Inhibited the Behavioral Effects of rrIL-1β

Social exploration. The duration of social exploration ranged from 100.5 to 114.2 s, and there was no difference in duration of social exploration between the different treatment groups prior to the initiation of the treatments (time 0) ($F(2,17) = 0.69$, $p = 0.52$).

A two-way ANOVA on the duration of social exploration with repeated measurements on the time factor revealed that duration of social exploration varied according to treatment and in a time-dependent manner (treatment: $F(2,17) = 12.63$, $p < 0.0001$; time: $F(4,68) = 16.68$, $p < 0.0001$; treatment $\times$ time $F(8,68) = 2.60$, $p < .05$). *Post hoc* comparisons of individual means by Fisher's test revealed that NBD peptide blocked rrIL-1β-induced decrease in social exploration 2–6 h after injection and recovery was complete after 24 h. NBD peptide had no effect of its own on the duration of social exploration in saline-treated animals (Figure 1).

Immobility

Saline-treated mice always remained active, which is why immobility data for these animals are not represented in Figure 1. Duration of immobility with repeated measurements on the time factor varied according to treatments

($F(1,14) = 17.07$, $p < 0.001$) but not to time factor and the interaction treatment $\times$ time was not significant (time: $F(2,28) = 2.71$, $p = 0.084$; treatment $\times$ time: $F(2,28) = 0.36$, $p = 0.70$).

Body Weight

Body weights did not differ according to treatments on time 0 (body weight (mean $\pm$ SEM): 305.0 ± 11.9 g in NBD peptide/saline-injected rat vs 324.1 ± 5.7 g in DMSO/rrIL-1β-injected rats vs 301.4 ± 7.3 g in NBD peptide/rrIL-1β-injected rats): ($F(2,17) = 2.3$, $p = 0.13$). Body weight changes with repeated measurements on the time factor differed according to treatment in a time-dependent manner (treatment: $F(2,17) = 10.3$, $p < 0.01$; time: $F(3,51) = 3.7$, $p < 0.05$; treatment $\times$ time: $F(6,51) = 3.8$, $p < 0.01$). *Post hoc* comparisons of individual means by Fisher's test revealed that NBD peptide attenuated rrIL-1β-induced body weight loss 2–24 h after injection compared to the injected group by DMSO/rrIL-1β. NBD peptide had no effect of its own on body weight loss (Figure 1).

Food Intake

Food intake with repeated measurements on the time factor differed according to treatment in a time-dependent manner

Figure 1 Effect of i.c.v. administration of NBD peptide on rrIL-1β-induced decrease of social exploration, food intake, body weight, and increase of immobility. NBD peptide (36 μg/2 μl) was injected i.c.v. 1 h prior to i.p. rrIL-1β (60 μg/kg), or saline. Data are expressed as mean $\pm$ SEM (number of rats are specified on each plot). (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, NBD peptide/rrIL-1β vs DMSO/rrIL-1β).

(treatment: $F(2,17)=10.9$, $p<0.001$; time: $F(3,51)=66.5$, $p<0.001$; treatment $\times$ time: $F(6,51)=9.5$, $p<0.001$). *Post hoc* comparisons of individual means by Fisher's test revealed that NBD peptide attenuated rrIL-1 β -induced decrease in food intake 2–24 h after injection. NBD peptide had no effect of its own on food intake in saline-treated animals (Figure 1).

NBD Peptide Inhibited c-Fos Activation Induced by rrIL-1 β

In order to test the effect of the NBD peptide on cellular activity, expression of c-Fos was measured in key immune responsive brain regions. Figure 2A shows the mean expression of c-Fos in the various brain structures of the rats in the different treatment groups.

Figure 2B shows a representative photomicrograph of the expression of c-Fos in the supraoptic nucleus (SON) for each treatment. rrIL-1 β induced a significant increase of c-Fos immunoreactivity in the nucleus of the tractus solitarius (NTS), paraventricular nucleus (PVN), SON, and lateral part of the central nucleus of the amygdala (CEAL). The effects of the main factors (pretreatment: DMSO or NBD peptide and treatment: NaCl or rrIL-1 β) and their interaction were significant in the PVN ($F(1,15)=5.47$; $p<0.05$), the CEAL ($F(1,16)=5.41$; $p<0.05$), and in the SON ($F(1,14)=16.9$; $p<0.001$). In the NTS, the effects of the main factors were significant but not their interaction. When allowed *Post hoc* comparisons of individual means by the Newman-Keuls test revealed that (1) NBD peptide had no effect of its own on c-Fos expression in CEAL and PVH but significantly increased it in SON compared to DMSO treatment, (2) rrIL-1 β significantly increased c-Fos expression, and (3) NBD peptide attenuated rrIL-1 β -induced c-Fos expression in PVN, CEAL, and SON (Figure 2b).

DISCUSSION

The main finding of the present study is that inhibition of NF κ B activation at the blood–brain interface prevented the establishment of behavioral depression normally induced by peripheral administration of IL-1 β and the neural activation of various brain structures already known to be responsive to peripheral IL-1 β .

The effect of an i.c.v. injection of NBD peptide has been evaluated in a recent work in order to demonstrate that this permeant inhibitor targets NF κ B activation at the blood–brain interface (Nadjar *et al*, in press). Even if mechanism of diffusion of an i.c.v. injected substance is not clearly elucidated, recent data by Mercier *et al* (2003) point out the evidence of a possible link between CSF compartment and perivascular cells via an extracellular matrix network (Mercier *et al*, 2003). Therefore, NBD peptide injected i.c.v. could travel through this extracellular matrix and consequently reach perivascular cells as demonstrated by injection and visualization of a biotinylated NBD peptide.

This study is the first to evaluate the involvement of the intracellular signaling NF κ B pathway in IL-1 β -induced sickness behavior. Even though the pivotal role of IL-1 β in cytokine-induced sickness behavior is well established (Kent *et al*, 1992; Bluthé *et al*, 1992b; Propes and Johnson,

1997), the postreceptor mechanisms involved in this phenomenon are still unknown. Various mediators dependent on NF κ B activation have already been shown to mediate the behavioral effects of IL-1, including prostaglandins (Bluthé *et al*, 1992a) and nitric oxide (Bluthé *et al*, 1992c). The rapid induction of NF κ B translocation that is observed at the blood–brain interface after IL-1 β fixation on its receptor (Nadjar *et al*, 2003) appears to be a crucial step in the induction of the behavioral effects of this cytokine. The blood–brain interface functions as a relay in the transmission of the peripheral immune message to the brain since it is there that proinflammatory cytokines and other inflammatory mediators are produced (Konsman *et al*, 2004, 2000b; Laflamme *et al*, 1999; Laflamme and Rivest, 1999; Nadjar *et al*, in press). The observation that administration of the NBD peptide, that block NF κ B activation at the level of the blood–brain interface, attenuates the behavioral effects of peripherally administered IL-1 β is consistent with this interpretation.

The results concerning the effects of the NBD peptide on c-Fos activation in the various brain structures under investigation are more difficult to interpret. Brain distribution of the expression of c-fos mRNA or c-Fos protein in response to peripheral IL-1 β injection has already been described in several previous studies. A common finding is induction of c-fos mRNA or c-Fos protein in cells of the PVH, the NTS, and several other neural structures such as the CEAL or the SON, 1 h after i.p. or i.v. injection of IL-1 β (Brady *et al*, 1994; Day and Akil, 1996; Ericsson *et al*, 1994; Herkenham *et al*, 1998). The preoptic area has also been reported to be activated (Brady *et al*, 1994), although this was not the case, at 1 h, in the present study. In terms of mechanisms, c-Fos activation of these various brain structures is assumed to be mediated neurally since it maps the primary and secondary projection areas of the vagus nerves (Konsman and Cartmell, 1997; Konsman *et al*, 2000a) and is abrogated by section of the vagus nerves under the diaphragm (Konsman *et al*, 2000a; Konsman and Dantzer, 2001).

The importance of the dorsal vagal complex (DVC) in social withdrawal that accompanies lipopolysaccharide (LPS) treatment was verified recently by Marvel *et al* (2004). The infusion of bupivacaine in the DVC decreased c-Fos expression in the NTS, SON, PVH, and CEAL in response to i.p. LPS and attenuated the LPS-induced sickness behavior. Part of Marvel's observation is fully compatible with the results of previous studies carried out on vagotomized animals. Indeed, the section of the vagus nerves under the diaphragm abrogates the induction of sickness behavior in response to peripheral administration of LPS (Bluthé *et al*, 1994) or IL-1 β (Bluthé *et al*, 1996a, b) while preserving response to i.c.v. administration of IL-1 β (Bluthé *et al*, 1996a). This is related to the fact that the DVC includes afferent of the vagus nerve that carries neural information (Swanson, 1998). However, the location in the DVC of a circumventricular organ, the area postrema, that receives humoral information also implies that the nerve connection between the CVO and the NTS (Shapiro and Miselis, 1985) plays an important role in the induction of sickness behavior because when it is abrogated by the bupivacaine treatment the humoral part of the peripheral signal remains inactive.

Figure 2 (A) Effects of NBD peptide on c-Fos protein synthesis induced by peripheral administration of rrIL-1β in the NTS, the SON, the CEAI, and the PVH. Bar graphs summarize the number of c-Fos-positive cells in the cerebral nuclei studied. Note that injection of rrIL1β significantly increases c-Fos immunoreactivity in the NTS (** $p < 0.01$), SON (*** $p < 0.001$), CEAI (*** $p < 0.01$), and PVH (** $p < 0.01$) compared to control groups and this effect is reversed by a preliminary i.c.v. injection of NBD peptide, in the SON and CEAI (** $p < 0.01$) and PVH (* $p < 0.05$). Data were analyzed by a two-way ANOVA and Newman-Keuls *post hoc* test (number of rats are specified on each plot). (B) Representative photomicrographs demonstrating the distribution of c-Fos immunoreactivity in the SON: (a) DMSO/NaCl, (b) DMSO/rrIL1β, (c) NBD/NaCl, and (d) NBD/rrIL1β. Scale bar = 100 μm. OC: optic chiasma.

According to their study, it could appear paradoxical that an intervention at the level of the blood–brain interface is able to attenuate a phenomenon, sickness behavior, that is supposed to be mediated by a mechanism bypassing the humoral pathway. The more rational explanation of this paradox would be the convergence of humoral and neural generated signals in brain structures, as the DVC, that are ultimately responsible for the behavioral effects of IL-1 β . According to this interpretation, the induction of c-Fos expression in the brain structures that are responsive to peripheral immune stimulation would require the combined effect of an afferent neural pathway and the local action of NF κ B-dependent mediators at the blood–brain interface. This does not need to take place in all the brain structures in which the neural pathway is projecting. The convergence could actually be limited to the primary projection area: the NTS. In our study, NTS is the only observed nuclei where NBD peptide treatment does not give clear cut results on c-Fos expression, although it significantly inhibits IL-1 β -induced Cox-2 synthesis (Nadjar et al, in press). Therefore, we hypothesize that impairment of one of the pathways (humoral or neural) will block the integrated signal in afferent structures of the DVC nuclei, as PVH, CEAL or SON and attenuate sickness behavior. The molecular basis of such integration needs further investigation in order to evaluate the real contribution of each pathway of communication.

In conclusion, NF κ B activation at the level of the blood–brain interface represents a crucial molecular event in neural activation and development of sickness behavior in response to a peripheral immune stimulus. Based on these findings, it can be speculated that NF κ B activation at the blood–brain interface represents a new target for the development of drugs that are able to alleviate the nonspecific symptoms of inflammation.

ACKNOWLEDGEMENTS

This research was supported by FRM (Fondation pour la Recherche Médicale), INRA (Institut National de la Recherche Agronomique), and CNRS (Centre National de la Recherche Scientifique). A Nadjar was supported by a studentship from the 'Ministère de l'Éducation Nationale, de la Recherche et de la Technologie'. We express our gratitude to S Poole (NIBSC, Potters Bar, UK) for providing the rrIL-1 β .

REFERENCES

- Anforth HR, Bluthé RM, Bristow A, Hopkins S, Lenczowski MJ, Luheshi G et al (1998). Biological activity and brain actions of recombinant rat interleukin-1 α and interleukin-1 β . *Eur Cytokine Netw* 9: 279–288.
- Baeuerle PA, Henkel T (1994). Function and activation of NF- κ B in the immune system. *Annu Rev Immunol* 12: 141–179.
- Bluthé RM, Castanon N, Pousset F, Bristow A, Ball C, Lestage J et al (1999). Central injection of IL-1 antagonizes the behavioural effects of lipopolysaccharide in rats. *Psychoneuroendocrinology* 24: 301–311.
- Bluthé RM, Crestani F, Kelley KW, Dantzer R (1992a). Mechanisms of the behavioral effects of interleukin 1. Role of prostaglandins and CRF. *Ann N Y Acad Sci* 650: 268–275.
- Bluthé RM, Dantzer R, Kelley KW (1992b). Effects of interleukin-1 receptor antagonist on the behavioral effects of lipopolysaccharide in rat. *Brain Res* 573: 318–320.
- Bluthé RM, Michaud B, Kelley KW, Dantzer R (1996a). Vagotomy attenuates behavioural effects of interleukin-1 injected peripherally but not centrally. *Neuroreport* 7: 1485–1488.
- Bluthé RM, Michaud B, Kelley KW, Dantzer R (1996b). Vagotomy blocks behavioural effects of interleukin-1 injected via the intraperitoneal route but not via other systemic routes. *Neuroreport* 7: 2823–2827.
- Bluthé RM, Michaud B, Kelley KW, Dantzer R (1997). Cholecystokinin receptors do not mediate the behavioral effects of lipopolysaccharide in mice. *Physiol Behav* 62: 385–389.
- Bluthé RM, Sparber S, Dantzer R (1992c). Modulation of the behavioural effects of interleukin-1 in mice by nitric oxide. *Neuroreport* 3: 207–209.
- Bluthé RM, Walter V, Parnet P, Laye S, Lestage J, Verrier D et al (1994). Lipopolysaccharide induces sickness behaviour in rats by a vagal mediated mechanism. *C R Acad Sci III* 317: 499–503.
- Brady LS, Lynn AB, Herkenham M, Gottesfeld Z (1994). Systemic interleukin-1 induces early and late patterns of c-fos mRNA expression in brain. *J Neurosci* 14: 4951–4964.
- Dantzer R (2001). Cytokine-induced sickness behavior: mechanisms and implications. *Ann N Y Acad Sci* 933: 222–234.
- Dantzer R (2004). Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. *Eur J Pharmacol* 500: 399–411.
- Dantzer R, Wollman EE, Vitkovic L, Yirmiya R (1999). Cytokines, stress, and depression. Conclusions and perspectives. *Adv Exp Med Biol* 461: 317–329.
- Dasgupta S, Jana M, Zhou Y, Fung YK, Ghosh S, Pahan K (2004). Antineuroinflammatory effect of NF- κ B essential modifier-binding domain peptides in the adoptive transfer model of experimental allergic encephalomyelitis. *J Immunol* 173: 1344–1354.
- Day HE, Akil H (1996). Differential pattern of c-fos mRNA in rat brain following central and systemic administration of interleukin-1 β : implications for mechanism of action. *Neuroendocrinology* 63: 207–218.
- Dinarello CA (2000). The role of the interleukin-1-receptor antagonist in blocking inflammation mediated by interleukin-1. *N Engl J Med* 343: 732–734.
- Ericsson A, Kovacs KJ, Sawchenko PE (1994). A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. *J Neurosci* 14: 897–913.
- Ericsson A, Liu C, Hart RP, Sawchenko PE (1995). Type 1 interleukin-1 receptor in the rat brain: distribution, regulation, and relationship to sites of IL-1-induced cellular activation. *J Comp Neurol* 361: 681–698.
- Gabay C, Kushner I (1999). Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 340: 448–454.
- Hayden MS, Ghosh S (2004). Signaling to NF- κ B. *Genes Dev* 18: 2195–2224.
- Herkenham M, Lee HY, Baker RA (1998). Temporal and spatial patterns of c-fos mRNA induced by intravenous interleukin-1: a cascade of non-neuronal cellular activation at the blood–brain barrier. *J Comp Neurol* 400: 175–196.
- Kent S, Bluthé RM, Dantzer R, Hardwick AJ, Kelley KW, Rothwell NJ et al (1992). Different receptor mechanisms mediate the pyrogenic and behavioral effects of interleukin 1. *Proc Natl Acad Sci USA* 89: 9117–9120.
- Konsman JP, Cartmell T (1997). Neural pathways from the immune system to the brain. International Workshop organized under the auspices of the BIO-MED I Concerted Action 'Cytokines in the brain.' Marseille, 7 December 1996. *Eur Cytokine Netw* 8: 221–223.

- Konsman JP, Dantzer R (2001). How the immune and nervous systems interact during disease-associated anorexia. *Nutrition* 17: 664–668.
- Konsman JP, Luheshi GN, Bluthé RM, Dantzer R (2000a). The vagus nerve mediates behavioural depression, but not fever, in response to peripheral immune signals; a functional anatomical analysis. *Eur J Neurosci* 12: 4434–4446.
- Konsman JP, Tridon V, Dantzer R (2000b). Diffusion and action of intracerebroventricularly injected interleukin-1 in the CNS. *Neuroscience* 101: 957–967.
- Konsman JP, Vigues S, Mackerlova L, Bristow A, Blomqvist A (2004). Rat brain vascular distribution of interleukin-1 type-1 receptor immunoreactivity: relationship to patterns of inducible cyclooxygenase expression by peripheral inflammatory stimuli. *J Comp Neurol* 472: 113–129.
- Krueger JM, Obal Jr F, Fang J (1999). Humoral regulation of physiological sleep: cytokines and GHRH. *J Sleep Res* 8(Suppl 1): 53–59.
- Laflamme N, Rivest S (1999). Effects of systemic immunogenic insults and circulating proinflammatory cytokines on the transcription of the inhibitory factor kappaB alpha within specific cellular populations of the rat brain. *J Neurochem* 73: 309–321.
- Laflamme N, Lacroix S, Rivest S (1999). An essential role of interleukin-1beta in mediating NF-kappaB activity and COX-2 transcription in cells of the blood-brain barrier in response to a systemic and localized inflammation but not during endotoxemia. *J Neurosci* 19: 10923–10930.
- Marvel FA, Chen CC, Badr N, Gaykema RP, Goehler LE (2004). Reversible inactivation of the dorsal vagal complex blocks lipopolysaccharide-induced social withdrawal and c-Fos expression in central autonomic nuclei. *Brain Behav Immunol* 18: 123–134.
- May MJ, D'Acquisto F, Madge LA, Glockner J, Pober JS, Ghosh S (2000). Selective inhibition of NF-kappaB activation by a peptide that blocks the interaction of NEMO with the IkappaB kinase complex. *Science* 289: 1550–1554.
- Mercier F, Kitasako JT, Hatton GI (2003). Fractones and other basal laminae in the hypothalamus. *J Comp Neurol* 455: 324–340.
- Miyamoto S, Verma IM (1995). Rel/NF-kappa B/I kappa B story. *Adv Cancer Res* 66: 255–292.
- Moynagh PN, Williams DC, O'Neill LA (1994). Activation of NF-kappa B and induction of vascular cell adhesion molecule-1 and intracellular adhesion molecule-1 expression in human glial cells by IL-1. Modulation by antioxidants. *J Immunol* 153: 2681–2690.
- Mulla A, Buckingham JC (1999). Regulation of the hypothalamo-pituitary-adrenal axis by cytokines. *Baillieres Best Pract Res Clin Endocrinol Metab* 13: 503–521.
- Nadjar A, Combe C, Laye S, Tridon V, Dantzer R, Amedee T et al (2003). Nuclear factor kappaB nuclear translocation as a crucial marker of brain response to interleukin-1. A study in rat and interleukin-1 type I deficient mouse. *J Neurochem* 87: 1024–1036.
- Nadjar A, Tridon V, May MJ, Ghosh S, Dantzer R, Amédée T et al (2005). NFkappaB activates *in vivo* the synthesis of inducible Cox-2 in the brain. *J Cereb Blood Flow Metab* 9 March [Epub ahead of print].
- O'Neill LA, Greene C (1998). Signal transduction pathways activated by the IL-1 receptor family: ancient signaling machinery in mammals, insects, and plants. *J Leukoc Biol* 63: 650–657.
- Propes MJ, Johnson RW (1997). Role of corticosterone in the behavioral effects of central interleukin-1 beta. *Physiol Behav* 61: 7–13.
- Sagar SM, Price KJ, Kasting NW, Sharp FR (1995). Anatomic patterns of Fos immunostaining in rat brain following systemic endotoxin administration. *Brain Res Bull* 36: 381–392.
- Shapiro RE, Miselis RR (1985). The central neural connections of the area postrema of the rat. *J Comp Neurol* 15: 344–364.
- Shu SY, Ju G, Fan LZ (1988). The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system. *Neurosci Lett* 85: 169–171.
- Swanson LW (1998). *Brain Maps: Structure of the Rat Brain* 2nd edn, Elsevier: Amsterdam.
- Turrin NP, Plata-Salaman CR (2000). Cytokine-cytokine interactions and the brain. *Brain Res Bull* 51: 3–9.