

IMMUNE CHALLENGE INDUCES DIFFERENTIAL CORTICOSTERONE AND INTERLEUKIN-6 RESPONSIVENESS IN RATS BRED FOR EXTREMES IN ANXIETY-RELATED BEHAVIOR

N. SALOME,^a A. TASIEMSKI,^b I. DUTRIEZ,^a A. WIGGER,^c R. LANDGRAF^c AND O. VILTART^{a,d,*}

^aDépartement Neurosciences et Physiologie Adaptatives, Université de Lille I, 59655 Villeneuve d'Ascq, France

^bLaboratoire de Neuroimmunologie des Annélides UMR 8017, Université de Lille I, 59655 Villeneuve d'Ascq, France

^cMax Planck Institute für Psychiatrie, 80804 München, Germany

^dLaboratoire de NeuroImmunoEndocrinologie, Institut Pasteur de Lille, IFR 142, 1, Rue Calmette, 59019 Lille, France

Abstract—Disturbances in mood such as anxiety and depression are often associated with altered hypothalamo-pituitary-adrenal (HPA) axis reactivity, but also with changes in cytokine production, such as interleukin-6 (IL-6), an essential immune factor produced by macrophages and lymphocytes during inflammatory processes. The reciprocal relationship between the HPA axis and the immune system is now well established. In order to understand better the endocrine reactivity of anxious individuals faced with an immune challenge, a model of innate anxiety-related behavior, HAB and LAB rats (HABs, high and LABs, low anxiety-related behavior) was used in this study. We sought to determine whether injection of lipopolysaccharide (LPS) induced a differential HPA axis reactivity and plasma IL-6 release in HABs and LABs.

After LPS injection, the plasma adrenal corticotrophic hormone increase did not differ between HABs and LABs, whereas a larger increase in plasma corticosterone levels occurred in HABs than in LABs at 2 h after injection. Moreover, basal IL-6 levels were lower in HABs than in LABs, leading to a higher IL-6 2 h/basal ratio in HABs. In conclusion, we propose for the first time a link between the endocrine and immune systems of HABs and LABs and suggest that IL-6 could be a neuroendocrine correlate of trait anxiety in HABs. © 2008 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: lipopolysaccharide, HPA axis, immune system, innate anxiety, HAB rats.

Stressful events are part of daily life. Even if the notion of “stress” remains poorly defined, it relates changes modifying our homeostasis to cope efficiently with a stressor. The stress response combines behavioral, autonomic and immune variations, together with the release of hormones

*Correspondence to: O. Viltart, Laboratoire de NeuroImmunoEndocrinologie, Institut Pasteur de Lille, IFR 142, 1, Rue Calmette, 59019 Lille, France. Tel: +33-0-3-20-87-12-44; fax: +33-0-3-20-87-12-33. E-mail address: odile.viltart@univ-lille1.fr (O. Viltart).

Abbreviations: ACTH, adrenal corticotrophic hormone; CRH, corticotrophin releasing hormone; HABs, high anxiety-related behavior rats; HPA, hypothalamo-pituitary-adrenal axis; IL, interleukin; KO, knockout; LABs, low anxiety-related behavior rats; LPS, lipopolysaccharide; non-REM, non-rapid eye movement.

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that form part of the hypothalamo-pituitary-adrenal (HPA) axis: corticotrophin releasing hormone (CRH), adrenal corticotrophic hormone (ACTH) and corticosterone. To date, a relationship between the HPA axis and the immune system is established, since peripheral or central injection of cytokines including interleukin- (IL-) 1 α , IL-1 β or IL-6 induce dose-dependent increases in plasma ACTH and corticosterone in rodents (Capuron and Dantzer, 2003). Furthermore, the cytokines released during systemic or localized inflammation influence behavioral outputs responsible for “sickness behavior” characterized by reduced social exploration, sexual behavior and food consumption (Dantzer et al., 2003). IL-6 has also been suggested to play a role in some CNS disorders including Alzheimer diseases, anorexia nervosa and depression (Pomeroy et al., 1994; Capuron and Dantzer, 2003; Quintanilla et al., 2004; Schiepers et al., 2005). In particular, physiological data emphasized the relationship between IL-6 and depression since peripheral injection of IL-6 in rodents induced increase in 5-HT metabolism, supported by an increase of brain tryptophan levels (Wang and Dunn, 1998). In addition, in humans and rodents, IL-6 was shown to be associated with sleep disturbances that are a common symptom in depressed patients (Späth-Schwalbe et al., 1998; Hogan et al., 2003). The use of mice with IL-6 knockout (KO) has underlined the potential anxiolytic effects of this cytokine (Armario et al., 1998; Butterweck et al., 2003; Chourbaji et al., 2006) whereas no clear-cut effect was obtained regarding its role in the modulation of depression-like behavior (Grippo et al., 2005; Chourbaji et al., 2006; Swiergiel and Dunn, 2006). Similarly, human data revealed contradictory results on IL-6 plasma levels in depressed patients both at basal levels and after antidepressant treatment (Frommberger et al., 1997; Maes et al., 1997; Basterzi et al., 2005). Elevated pro-inflammatory IL-6 plasma levels were described in nontreated depressed patients (Frommberger et al., 1997; Maes et al., 1997), whereas no changes can be also observed on baseline for patients showing major depression (Basterzi et al., 2005). Thus, changes observed in the plasma levels of IL-6 of depressed patients might be due to the severity of the depression. IL-6 also participates in the metabolism and energy homeostasis (Wallenius et al., 2002a,b; Holmes et al., 2004). Increases in the secretion of IL-6 were observed both in obese and anorectic subjects (Raymond et al., 2000; Kahl et al., 2004) and immunological dysfunction described in patients exhibiting such a chaotic eating behavior seems to be associated with a depressive and/or anxious state. In fact, participants with bulimia nervosa presented the lowest

lymphocyte proliferative responses that were negatively correlated with anxiety trait but were enhanced with improvement of the bulimic state (Nagata et al., 2006).

The use of adequate animal models expressing extremes in anxiety- and depression-like behaviors enables the investigation of the different processes linking neuroendocrine and immune systems with behavior. Growing behavioral and physiological studies have shown that the high (HABs) and low (LABs) anxiety-related behavior rats, two Wistar rat lines selectively bred for their opposite behavioral performances in the elevated plus-maze test (Liebsch et al., 1998), are considered as valuable and robust models of innate anxiety- and depression-like behaviors (Salomé et al., 2002; Landgraf et al., 2007). In particular, HABs show signs of a differential HPA axis upon stressor exposure and an altered response to the dexamethasone (DEX)/CRH test (Landgraf et al., 1999; Keck et al., 2002; Frank et al., 2006). However, until now, no study has examined the reactivity of their HPA axis following an immune challenge.

Thus, considering the close relationship between emotional reactivity, the HPA axis and the immune system, the aim of this study was to determine whether an immune challenge may differentially affect the HPA axis and the plasma IL-6 response in HABs and LABs.

EXPERIMENTAL PROCEDURES

Animals

The experiments were approved by the Institutional Animal Care and Use Committee in accordance with the principles of laboratory animal care (European Communities Council Directive 24/111986; 86/609/EEC) and followed the Institute for Laboratory Animal Research "Guide for Care and Use of Laboratory Animals." All procedures have been carried out in order to minimize the number of animals used and their suffering. The adult male rats (12 weeks old) used in this study, HABs and LABs, have been selected and bred at the Max Planck Institute of Psychiatry (Munich, Germany). The data presented in this paper were obtained from animals of the F10–F12 generation.

Animals were housed in groups of up to five individuals (cage size: 58×38×20 cm). They were maintained on a 12-h light/dark cycle (lights on 6:00 am) with food and water available *ad libitum*. At least 2 h before testing, the animals were brought to the testing room. All lipopolysaccharide (LPS) injections were done between 9:00 am and 10:00 am.

Surgical procedure

Five days prior to the experiments, nine HABs and nine LABs were weighed and deeply anesthetized with halothane (Sigma-Aldrich, Munich, Germany), evaluated by an absence of response following toe pinch and eye blink, for implantation of a catheter in the jugular vein. The jugular vein was exposed and a Silastic tubing catheter (i.d. 0.025 inch, o.d. 0.047 inch, Dow Corning, Midland, MI, USA), connected to PE-50 polyethylene tubing, was inserted into the vessel with the tip positioned at the right atrium (Neumann et al., 1998). The catheter was passed s.c., exteriorized dorsally in the cervical region and plugged by a steel insert. The ventral wound was closed with sterile surgery clips. The catheter was filled with sterile 0.9% saline containing gentamicin (30 kIU/ml; Centravet, Bad Bentheim, Germany) and flushed with the same solution 3 days later. Following surgery, rats were housed individually in experimental cages allowing subsequent

blood sampling from the freely moving animals during the experiment.

Blood samplings and i.p. LPS injection

On day 5 after surgery at 7:30 am, the catheters were attached to an extension tubing connected to a 1-ml plastic syringe filled with sterile saline containing heparin (30 IU/ml, Heparin-Natrium, Ratiopharm, Ulm, Germany), and the rats were then left undisturbed for 90 min. A 0.5 ml blood sample was removed from freely moving rats of each line under basal resting conditions (basal group), in order to measure the plasma levels of ACTH, corticosterone and IL-6. Blood was immediately substituted by injection of 0.9% saline to each rat, after each sampling. After 90 min rest in their home cage, rats were injected with LPS (*Salmonella abortus equi*, serotype No. L-6636; Sigma, dissolved in pyrogen-free saline, 30 µg/kg, i.p.). Further blood samples were taken 2 h and 4 h after the injection. At the end of the experiment, the catheters were flushed with gentamicin solution and plugged.

Hormone assays

All blood samples were collected in ice-cooled polyethylene tubes containing ethylene diamine tetraacetic acid (100 µl, 5% EDTA) and aprotinin (Trasylo[®], Bayer, Leverkusen, Germany) to prevent clotting. The samples were immediately centrifuged at 4 °C (4000×g, 5 min). Plasma aliquots were made: 80 µl for ACTH assay, 30 µl for corticosterone assay, 50 µl for IL-6 assay. Each aliquot was stored at –20 °C, except for those for ACTH, which were stored at –80 °C.

Plasma ACTH and corticosterone levels were measured using commercially available radioimmunoassay kits (ACTH: Biochem Immunosystems, Freiburg, Germany, sensitivity <1 pg/ml; corticosterone: DRG-Instruments, Marburg/Lahn, Germany, sensitivity <2 pg/ml) according to the respective protocols. Finally, the plasma IL-6 levels were determined using an immunoassay kit (ELISA, Biosource International, Inc., Camarillo, CA, USA, sensitivity <8 pg/ml).

[¹²⁵I] (Phe²,NLe⁴) ACTH (1–24) binding to ACTH receptors

In another group of rats, five HABs and seven LABs were decapitated 2 h after LPS injection and adrenals were removed. Receptors were quantified on individual extracts of right adrenals as previously described (Chatelain et al., 2003). Tissues were homogenized in 2.0 ml of ice-cold buffer (50 mM Tris–HCl, 100 mM NaCl and 6 mM MgCl₂, pH 7.4) and homogenates were centrifuged at 1000×g for 20 min at 4 °C. The supernatant was collected, sedimented at 20,000×g (10 min at 4 °C) and washed once in new buffer and re-centrifuged. The final pellet was re-suspended in the homogenization buffer at a protein concentration of 1 mg/ml and aliquots were stored at –80 °C. Assays were performed by incubating membranes (1 mg of protein/ml) in ice-cold buffer (50 mM Tris–HCl, 100 mM NaCl, 6 mM MgCl₂, 1 µg/ml aprotinin and 0.1% BSA, pH 7.4) with 50 pM of [¹²⁵I](Phe²,NLe⁴)ACTH (1–24). Saturation studies were performed with various concentrations (0.01–100 nM) of (Phe²,NLe⁴)ACTH (1–24) (Sigma, Saint-Quentin Fallavier, France). All samples were incubated 20 min at 4 °C. Separation of bound from free [¹²⁵I](Phe²,NLe⁴)ACTH (1–24) was performed by rapid filtration through 0.2% polyethylenimine pre-treated GF/B glass-fiber filters (Whatman Int. Ltd., Madistone, UK) using a Millipore cell harvester and three ice-cold buffer washes (pH 7.4). Non-specific binding was determined in the presence of 1 µM (Phe²,NLe⁴)ACTH (1–24). Under such conditions, non-specific binding corresponded to 10% of total [¹²⁵I](Phe²,NLe⁴)ACTH (1–24) binding. Protein concentrations were determined using RC DC Protein Assay (BIORAD), with BSA as standard. B_{max} and K_d values were calculated from Scatchard plots.

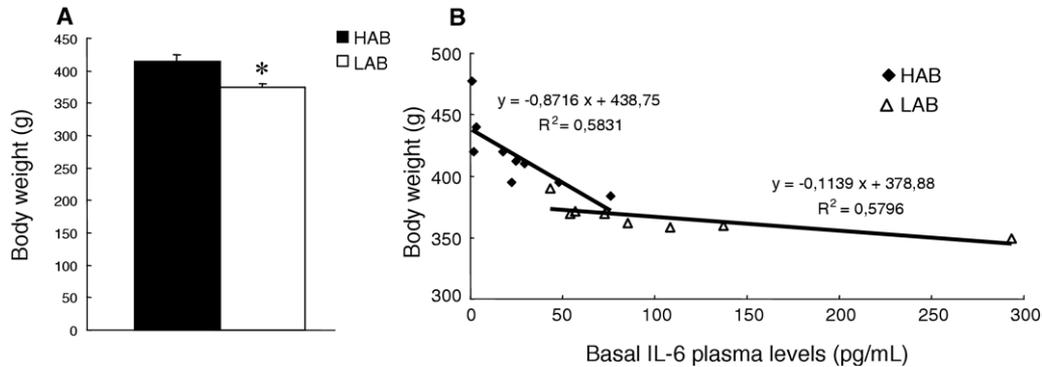


Fig. 1. (A) Comparison of body weight of the 12-week-old HABs ($n=9$) and LABs ($n=9$). (B) Linear correlation plots for plasma IL-6 levels and HABs (full quadrilateral) and LABs (open triangle) body weight. Values are expressed as mean \pm S.E.M. * $P < 0.05$.

Statistical analysis

Data are expressed as mean \pm standard error of the mean (S.E.M.). Basal levels and ratios (i.e. values obtained at different times divided by the baseline levels) of plasma ACTH, corticosterone and IL-6 levels, as well as binding ACTH parameters, were compared using Student's tests for unpaired values. One-way (line \times time) ANOVA for repeated measures followed by Scheffé post hoc test was used to analyze changes in plasma ACTH, corticosterone and IL-6 levels.

RESULTS

Animal weights

Using age-matched groups of adult animals we found that, on the day of the experiment, the weight of HABs was higher than that of the LABs ($t_{16}=3.26$, $P < 0.005$; Fig. 1A).

Plasma ACTH levels

Basal plasma ACTH levels were not different between HABs and LABs ($t_{16}=-1.40$, n.s., Table 1). ANOVA analysis revealed a main time effect ($F_{(2,32)}=114.37$, $P < 0.001$), irrespective of lines, since LPS injection increased plasma ACTH levels with the highest levels 2 h after injection ($P < 0.001$). In addition, the plasma levels

were higher at 2 h than at 4 h after LPS injection ($P < 0.001$). ANOVA analysis did not support any significant line effect ($F_{(1,16)}=0.49$; n.s., Table 1) nor time \times line interaction ($F_{(2,32)}=0.38$; n.s., Table 1). Finally, the 2 h/basal ($t_{16}=0.28$, n.s.) and 4 h/basal ratios ($t_{16}=0.52$, n.s.) were not different between HABs and LABs (Table 1).

Plasma corticosterone levels

Basal plasma corticosterone levels were not different between HABs and LABs ($t_{16}=0.87$, n.s.). ANOVA analysis revealed a main time effect ($F_{(2,32)}=97.91$; $P < 0.001$, Table 1), irrespective of lines, since LPS injection induced an increase in plasma corticosterone levels measured both at 2 h ($P < 0.001$) and at 4 h ($P < 0.001$) after injection compared with basal levels, as well as higher plasma corticosterone levels at 2 h than at 4 h after LPS injection ($P < 0.001$). Moreover, ANOVA analysis revealed a main lines effect ($F_{(2,32)}=9.7$; $P < 0.01$), irrespective of time, as HABs displayed higher plasma corticosterone levels than LABs. ANOVA analysis revealed also a significant time \times line interaction ($F_{(2,32)}=5.58$; $P < 0.01$, Table 1) indicating that both HABs and LABs exhibited an increase in their plasma corticosterone levels at 2 h and 4 h after LPS

Table 1. Plasma ACTH, corticosterone and IL-6 levels, and ratios for plasma ACTH, corticosterone and IL-6 levels calculated from individual values obtained at 2 h (2 h/basal) and at 4 h (4 h/basal) of male HABs and LABs in basal condition (basal), at 2 h and 4 h after lipopolysaccharide (*Salmonella abortus equi*) injection

	Basal	2 h	4 h	Ratio 2 h/basal	Ratio 4 h/basal	Time	Line	Interaction
ACTH (pg/ml)								
HAB	45.3 \pm 6.10	2129 \pm 216.6	250.1 \pm 87.0	56.0 \pm 9.1	7.0 \pm 3.4	$P < 0.001$	n.s.	n.s.
LAB	59.7 \pm 8.20	2389 \pm 322.9	285.1 \pm 59.8	50.9 \pm 15.5	5.2 \pm 0.9			
Corticosterone (ng/ml)								
HAB	45.9 \pm 12.8 ^{°°}	418.9 \pm 17.8 ^{**}	251.7 \pm 48.2 ^{###}	15.4 \pm 3.2	8.6 \pm 2.4	$P < 0.001$	$P < 0.001$	$P < 0.001$
LAB	32.9 \pm 7.80 ^{°°}	273.6 \pm 11.7	231.9 \pm 8.80	14.4 \pm 4.2	12.2 \pm 3.6			
IL-6 (pg/ml)								
HAB	28.5 \pm 8.80 [*]	3908 \pm 670.9	726.9 \pm 295.1	362 \pm 110.2 [*]	89.8 \pm 32.1	$P < 0.001$	n.s.	n.s.
LAB	91.7 \pm 28.9	4891 \pm 425.5	775.4 \pm 170.1	75.0 \pm 18.2	15.3 \pm 5.4			

Values are expressed as mean \pm S.E.M. HABs, $n=9$ per group; LABs, $n=8-9$ per group.

* $P < 0.05$ vs. LABs for the same parameter.

** $P < 0.01$ vs. LABs for the same parameter.

°° $P < 0.01$ basal vs. 2 h and 4 h for the same line.

$P < 0.01$ HAB 2 h vs. HAB 4 h.

Table 2. Adrenal ACTH receptor characteristics of male HABs ($n=5$) and LABs ($n=7$), 2 h after lipopolysaccharide (*Salmonella abortus equi*) injection

	High-affinity sites		Low-affinity sites	
	K_d (nM)	B_{max} (fmol/mg)	K_d (nM)	B_{max} (fmol/mg)
HAB	2.3±0.3	90.8±12.3	0.11±0.04	9.2±0.9
LAB	1.7±0.3	78.5±12.8	0.05±0.03	12.2±2.7

Values are expressed as mean±S.E.M. HABs, $n=5$; LAB, $n=7$.

injection ($P<0.001$, Table 1). The corticosterone levels measured 2 h after the immune challenge were higher in HABs than in LABs ($P<0.001$, Table 1). Furthermore, only the HABs' corticosterone levels were higher at 2 h than at 4 h after the LPS injection ($P<0.001$, Table 1). Finally, the 2 h/basal and 4 h/basal ratios were not different between the two lines ($t_{16}=0.17$, n.s.; $t_{16}=0.85$, n.s., respectively, Table 1).

Plasma IL-6 levels

The basal plasma levels of IL-6 were significantly lower in HABs than in LABs ($t_{15}=-2.48$; $P<0.05$) and negatively correlated with the animals' weights ($r=-0.76$, $P<0.05$, Fig. 1B). ANOVA analysis revealed a main time effect ($F_{(2,32)}=95.26$, $P<0.001$, Table 1), irrespective of lines, since LPS injection resulted in a significant increase of plasma IL-6 ($F_{(2,32)}=95.26$, $P<0.001$) measured at 2 h and at 4 h after injection ($P<0.001$). In addition, plasma levels of IL-6 at 2 h were higher than at 4 h after the injection ($P<0.001$). ANOVA analysis did not suggest any significant line effect ($F_{(1,16)}=1.54$; n.s.) nor time×line interaction ($F_{(2,32)}=1.33$; n.s.). Interestingly, the 2 h/basal ratio was higher in HABs than in LABs ($t_{15}=2.42$; $P<0.05$) whereas the 4 h/basal ratio tended to be higher in HABs relative to LABs ($t_{15}=1.94$, $P=0.07$).

Adrenal ACTH binding

The K_d and B_{max} were not significantly different between HABs and LABs both for the high (H) and low (L) affinity sites (Table 2; K_d H: $t_{10}=1.20$, n.s.; K_d L: $t_{10}=1.18$, n.s.; B_{max} H: $t_{10}=0.63$, n.s.; B_{max} L: $t_{10}=-0.87$, n.s.).

DISCUSSION

This study provides the first evidence that 1) HABs and LABs display differential HPA axis and IL-6 responses after an immune challenge and 2) HABs have lower basal plasma IL-6 levels than LABs. In agreement with previous studies (Landgraf et al., 1999; Salomé et al., 2006), we confirmed the absence of a basal difference in ACTH and corticosterone plasma levels. Moreover, as previously reported in Wistar rats, LPS injection (*Salmonella abortus equi*), considered as a physiological stressor (Anisman and Matheson, 2005), increased both plasma ACTH and corticosterone levels (Mathias et al., 1999). These rises in ACTH and corticosterone were also reported in rodent models with other types of LPS (Lenczowski et al., 1997; Bethin et al., 2000). Here, the LPS injection induced higher

corticosterone levels in HABs than LABs, whereas plasma ACTH levels increased to similar levels in both lines. The ACTH reactivity differed from those obtained after a psychological stressor, including exposure to an open arm of the elevated plus-maze. In this condition, in comparison to LABs, HABs displayed higher plasma ACTH levels that could be associated with their higher plasma corticosterone levels (Landgraf et al., 1999; Salomé et al., 2006; Frank et al., 2006). Thus, the HPA reactivity of HABs and LABs may depend upon the nature of the stressor. In our study, the higher increase in HABs' plasma corticosterone levels compared with LABs associated with similar changes in the plasma ACTH concentrations did not appear to be the consequence of a difference in adrenal ACTH receptors sensitivity, since no alteration in the binding properties of the adrenal ACTH receptors was observed in either line after LPS injection. However, variations in plasma levels of IL-6 after LPS injection were associated with increased ACTH and corticosterone levels. Thus, one can speculate that this association may be an alternative explanation for the corticosterone changes observed in our study (Givalois et al., 1994; Wang and Dunn, 1999). Indeed, even if HABs and LABs showed a similar increase in plasma IL-6 levels after LPS injection, HABs displayed a higher 2 h/basal ratio than LABs that can result from their low basal plasma IL-6 levels. Moreover, the well-known effect of IL-6 to increasing plasma corticosterone levels (Lenczowski et al., 1997; Bethin et al., 2000) supports the hypothesis that the higher reactivity of IL-6 in HABs might play a pivotal role in the altered corticosterone response observed after an immune challenge. The origin and the consequence of this difference in plasma IL-6 levels between HABs and LABs remain to be determined. However, peripheral injection of IL-6 increases ambulatory exploration, locomotion and rearing in a new environment (Zalcman et al., 1998). In addition, IL-6 KO mice show higher anxiety-like behavior than their wild type counterparts in the elevated plus-maze as well as in the dark-light box (Armario et al., 1998; Chourbaji et al., 2006). Hence, these data support that endogenous IL-6 might exert anxiolytic effects. However, Swiergiel and Dunn (2006) failed to obtain an increase of anxiety-related behavior in IL-6 KO mice and recently showed an anxiogenic effect of LPS injection that has been related to a decrease of general activity (Swiergiel and Dunn, 2007). Concerning the HABs and LABs, their anxious phenotype was demonstrated to be robust in several models of unconditioned anxiety (Landgraf and Wigger, 2003). In addition, HABs displayed less locomotor activity and rearing when faced to novel and stressful situations (Landgraf and Wigger, 2003). Thus, the difference in basal plasma IL-6 levels between HABs and LABs could be related to their different emotionality. Furthermore, the behavior of group-housed HAB and LAB rats observed under basal conditions in their home cages was different since HAB rats present more sleeping/resting than their LAB counterparts (Henniger et al., 2000). Because of the existing relation between IL-6 and sleep, a physiological process often altered in anxiety and major depression, this observation is of particular interest. In fact,

in human, daytime plasma levels of IL-6 are inversely correlated with the amount of nighttime sleep, and sleep deprivation increases plasma levels of IL-6 (Shearer et al., 2001; Vgontzas et al., 1997, 1999). In rats, central injection of IL-6 altered the non-rapid eye movement (non-REM) sleep similarly to what it has been reported in humans after a subcutaneous IL-6 injection (Späth-Schwalbe et al., 1998). In HABs and LABs, Lancel and coworkers (2002) reported that these two lines exhibited a different spontaneous sleep-wake, with HABs spending less time in wakefulness and more time in non-REM than LABs. These results support the inverse association between low plasma levels of IL-6 found here and their prolonged sleep period.

Beside the IL-6 effects on anxiety-related behavior, this cytokine is hypothesized to be a key factor in the mechanisms involved in the pathogenesis of depression. However, results obtained from animal and human studies are often inconsistent. As an example, in IL-6 KO mice, the reduced behavioral despair in the forced swim and tail suspension tests, and an enhanced hedonic behavior in the saccharin test obtained by Chourbaji et al. (2006) could not be reproduced in other studies (Anisman and Merali, 2002; Swiergiel and Dunn, 2006). Even if some human studies reported increased plasma IL-6 levels in non-treated depressed patients (Maes et al., 1995; Frommberger et al., 1997), others have shown opposing results (Haack et al., 1999; Marques-Deak et al., 2007) often generated by confounding factors such as the age of the patients or the status of smokers (Haack et al., 1999). Moreover, the effects of antidepressants on IL-6 levels led to controversial results (Sluzewska et al., 1995; Frommberger et al., 1997; Maes et al., 1997; Kubera et al., 2000; Basterzi et al., 2005). Indeed, an increase in serum IL-6 levels has been reported in depressed patients treated with selective 5-HT reuptake inhibitors or lithium (Haack et al., 1999; Kubera et al., 2004; Marques-Deak et al., 2007). Likewise, in rats, administration of 5-HT reuptake inhibitors induced an increase of splenocyte IL-6 production (Kubera et al., 2000). These authors suggested that the therapeutic activity of these antidepressants was at least partly associated with the IL-6 homeostasis. In this context, IL-6 may thus interfere with the serotonergic system by stimulating 5-HT release and increasing 5-HT turnover (Calapai et al., 2001; Zhang et al., 2001; Lenard and Dunn, 2005). Interestingly, the HABs exhibited a higher depression related-behavior than LABs and a deficiency in the 5-HT neurotransmission and metabolism (Umriukhin et al., 2002; Landgraf and Wigger, 2003; Keck et al., 2005; Salomé et al., 2006). Thus, we cannot exclude the possibility that the modified basal plasma IL-6 levels we observed in HABs may be primarily related to either depression like-behavior and/or altered 5-HT system.

Finally, the difference in basal IL-6 levels found in HABs could also be related to another physiological process that involves this cytokine. In our study, we found a negative correlation between basal plasma levels of IL-6 and the weight of HABs and LABs. In fact, IL-6 is strongly involved in the regulation of energy homeostasis, particu-

larly in lipolysis, energy expenditure, decreased appetite and weight gain (Stouthard et al., 1995; Finck and Johnson, 1997; Wallenius et al., 2002a,b; Holmes et al., 2004). Indeed, circulating levels of IL-6 are elevated in obese humans and animals (Roytblat et al., 2000; Harkins et al., 2004). Furthermore, IL-6 deficient mice developed mature-onset obesity at 6–7 months of age, and peripheral treatment was able to partly reverse obesity (Wallenius et al., 2002a). These obese mice showed an increased synthesis of acylation-stimulating proteins (Wernstedt et al., 2006) which stimulated the uptake of glucose and lipids into adipose tissue (Murray et al., 1999; Xia et al., 2002) and whose levels were decreased after IL-6 treatment (Wernstedt et al., 2006). Altogether, these data support the relation between low basal plasma IL-6 levels displayed by HABs and their higher body weight compared with LABs.

CONCLUSION

In conclusion, the data we obtained with the HABs and LABs highlight a link between immune alterations and HPA dysfunction as usually observed in various psychiatric diseases. Furthermore, this study reinforces the usefulness of the HAB/LAB rat model to assess not only the neurobiological changes occurring after exposure to psychological stressors, but also to analyze the neuronal basis of HPA reactivity and physiological immune mechanisms after an immune challenge. The alterations described in this study suggest that IL-6 could be a neuroendocrine correlate of trait anxiety in HABs and provide a new window to investigate the field of psycho-immuno-neuroendocrinology with special regard to the physiological origins of anxiety-related and depression-like behavior in relation to metabolic regulation.

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