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The authors have studied mechanisms which could be involved in the sustained activation of the hypothalamus–pituitary–adrenal (HPA) axis during continuous infusion of rats with recombinant human interleukin-1β (IL-1β). First, the effects of 3 days of intracerebroventricular (i.c.v.) infusion of rats with IL-1 on plasma adrenocorticotropin (ACTH) and corticosterone (B) levels were investigated. Thereafter, changes in plasma ACTH and B levels were followed in rats intraperitoneally (i.p.) infused with IL-1β after immunoneutralization of corticotropin-releasing hormone (CRH), hypophysectomy (HPX), macrophage depletion using dichloromethylene diphosphonate (Cl2MDP)-containing liposomes, adrenalectomy (ADX) and dexamethasone (DEX) administration, respectively. Infusion of IL-1β i.c.v., even in doses as low as 0.1 μg/day, induced significant increases in plasma ACTH and B levels. HPX and ADX rats died within 18 h after starting the IL-1β infusion (0.5 μg/day). Immunoneutralization of CRH significantly decreased and macrophage depletion significantly increased the stimulation of the HPA axis by IL-1β (4.0 μg/day). Administration of high doses of DEX completely abolished the stimulation of the HPA axis by IL-1β (2.0 μg/day). The present study demonstrates that lower doses of IL-1β were able to activate the HPA axis when infused intracerebroventricularly compared with intraperitoneally. Regarding stimulation of the HPA axis by chronic i.p. infusion of IL-1β the present study: (1) provides evidence that the CRH system is involved; (2) provides no evidence for a direct stimulatory effect of IL-1β on the release of B by the adrenal gland which is of sufficient magnitude to resist the stress of chronic i.p. IL-1β infusion; (3) shows that endogenous macrophage-derived mediators, induced by i.p. IL-1β infusion, express an overall inhibitory rather than a stimulatory effect on the activity of the HPA axis; (4) demonstrates that exogenous administration of DEX blocks the effect of IL-1β, which fits well in the concept of an immunoregulatory feedback between IL-1β and glucocorticoids.

There is increasing evidence that bidirectional communication between the immune system and the neuroendocrine system enables an organism to respond appropriately to disturbances of its homeostasis by infection and inflammation. During these circumstances activation of the hypothalamus–pituitary–adrenal axis (HPA axis) is of utmost importance and interleukin-1β (IL-1β), a polypeptide mainly produced by macrophages, is thought to be a mediator of this activation. Increased levels of IL-1β have been measured in biological fluids of patients suffering from some infectious or inflammatory diseases, most probably indicating chronically elevated IL-1β production during these circumstances. In this context we have previously shown that continuous intraperitoneal (i.p.) infusion of rats with IL-1β (2 and 4 μg/day) for 7 days induced sustained activation of the HPA axis, an increase in rectal temperature and a decrease in food intake.

In the present study we have extended our earlier observations on chronic IL-1β infusion in rats and we have investigated some potential mechanisms which could be involved in the activation of the HPA axis during 3 days of continuous infusion with IL-1β. First, the authors investigated whether, like chronic i.p. administration of IL-1β, chronic central infusion of IL-1β is also able to activate the HPA axis. Therefore, IL-1β was infused for 3 days into the cerebroventricular...
ular system in doses lower than in our i.p. infusion studies. 6 Second, we investigated the involvement of the corticotropin-releasing hormone (CRH) system in the activation of the HPA axis during continuous i.p. infusion by IL-1β by pretreatment of the rats with a monoclonal anti-CRH antibody. Third, whether peripheral mechanisms, such as release of mediators from IL-1β-activated macrophages or a direct effect of IL-1β on corticosterone (B) production by the adrenal glands, are involved in the activation of the HPA axis during continuous i.p. infusion of IL-1β was investigated. Therefore, IL-1β was chronically infused i.p. in macrophage-depleted rats and in hypophysectomized rats. Finally, whether high or low concentrations of circulating glucocorticoids are capable of modulating the effects of continuously infused IL-1β was examined.

For this purpose IL-1β was infused i.p. in rats treated with high doses of the synthetic glucocorticoid dexamethasone and in adrenalectomized rats. Plasma ACTH and B levels were measured in blood samples which were withdrawn daily by means of a chronic jugular cannula. In addition, we examined in all experiments the effects of the different (pre)treatments of rats on rectal temperature, body weight change, and on food and fluid intake.

RESULTS

Infusion (both peripheral and central) of IL-1β induced clear signs of physical discomfort to the animals, including piloerection and decreased physical activity, starting a few hours after implantation of the pumps. This visually observed uneasiness gradually diminished during the first day of infusion and disappeared on day 2.

Effects of intracerebroventricular infusion of rats with IL-1β

Figure 1 shows the effects of 3 days continuous i.c.v. infusion of saline or IL-1β (100 and 500 ng IL-1β/day) on plasma levels of ACTH and B, rectal temperature, daily body weight change and on food and fluid intake. Continuous infusion of 500 ng IL-1β/day induced a significant increase in plasma ACTH levels. Due to shortage of plasma, ACTH levels of animals infused with 100 ng IL-1β/day could not be measured. Intracerebroventricular infusion of IL-1β induced a dose-dependent increase (P < 0.0005) in plasma B levels (AUC) and a dose-dependent increase (P < 0.0005) in total adrenal weight (saline: 44.8 ± 0.7 mg; 100 ng IL-1β: 46.7 ± 1.3 mg; 500 ng IL-1β: 57.3 ± 2.2 mg; mean ± SEM of 10–11 rats). Dunnett's t-test revealed that the adrenal weight of rats infused with the highest dose of IL-1β was significantly higher than that of saline-infused rats (P < 0.05). Rectal temperature increased dose-dependently (P < 0.0005) in response to chronic i.c.v. infusion of IL-1β (AUC). Continuous i.c.v. infusion of IL-1β induced a dose-dependent decrease in body weight gain and also in food and fluid intake (AUC; all three: P < 0.0005).

Effects of pretreatment with anti-CRH antiserum in rats infused i.p. with IL-1β or saline

The effects of pretreatment of rats with anti-CRH antiserum on the earlier mentioned parameters in rats infused i.p. with IL-1β (4.0 µg/day) or saline are shown in Figure 2. Pretreatment of rats with the anti-CRH antibody induced a small, though not significant decrease in plasma B levels compared with NRI pretreatment. Continuous infusion of 4.0 µg IL-1β/day induced a significant increase in plasma ACTH and B levels compared with infusion of saline. The IL-1β-induced increase in total adrenal weight was not significantly decreased by pretreatment of the rats with the anti-CRH antiserum (P < 0.05), whereas the IL-1β-induced plasma B response was slightly, but not significantly diminished. The IL-1β-induced increase in total adrenal weight was not significantly decreased by pretreatment of the rats with the anti-CRH antibody (55.3 ± 5.4 mg vs 52.7 ± 4.4 mg, NRI/IL-1β vs anti-CRH/IL-1β, NS). Rectal temperature was significantly increased by IL-1β compared with saline infusion (P < 0.05). Treatment with anti-CRH antiserum prior to saline infusion had no significant effect on rectal temperature nor did treatment with anti-CRH antiserum significantly affect the IL-1β-induced increase in rectal temperature. Continuous infusion of 4.0 µg IL-1β/day for 3 days significantly decreased body weight gain, and food and fluid intake compared with values in saline-infused animals. Treatment of rats with anti-CRH antiserum prior to saline infusion had no effect on these three parameters nor did it significantly affect the IL-1β-induced decreases in body weight gain and food intake. The IL-1β-induced decrease in fluid intake, however, was significantly larger in the group of animals pretreated with anti-CRH antiserum compared with that of animals pretreated with NRI (P < 0.05).

Effects of macrophage depletion or hypophysectomy (HPX) in rats infused i.p. with IL-1β or saline

The effects of macrophage depletion by pretreatment of rats with liposomes containing dichloromethylene diposphonate (Cl-MDP) on the earlier mentioned parameters in rats infused i.p. with IL-1β (4.0 µg/day) or saline are shown in Figure 3. Pretreatment of rats with Cl-MDP-liposomes had no significant effect on plasma ACTH and B levels and on total adrenal weight compared with values of control rats. Continuous i.p. infusion of 4.0 µg IL-1β/day
induced a significant increase in plasma ACTH and B levels compared with saline infusion. Pretreatment of rats with Cl₂MDP-liposomes significantly raised the IL-1β-induced increase in plasma ACTH and B levels (both \( P < 0.05 \)). Pretreatment of the rats with Cl₂MDP-liposomes induced a slight, although not significant, increase in the IL-1β-induced increase in total adrenal weight (55.8 ± 1.3 mg vs 63.4 ± 2.7 mg, saline/IL-1β vs Cl₂MDP-liposomes/IL-1β, NS). IL-1β infusion induced a significant increase in rectal temperature. Pretreatment with Cl₂MDP-liposomes slightly, though not significantly, increased rectal temperature of saline-infused rats, and it had no significant effect on the IL-1β-induced increase in rectal temperature. Continuous infusion of 4.0 μg IL-1β/day significantly decreased body weight gain, and food and fluid intake as compared to control values. Treatment of rats with Cl₂MDP-liposomes prior to saline infusion slightly, though not significantly, decreased these three parameters, and it also did not significantly affect the IL-1β-induced decrease in body weight gain, food and fluid intake.

All of the HPX rats infused with 0.5 μg IL-1β/day had died within 18 h after pump implantation, whereas sham-operated rats did not die during IL-1β infusion.

Figure 1. Effects of 3 days continuous intracerebroventricular (i.c.v.) infusion of rats.

With saline (●) or increasing doses of recombinant human IL-1β [100 ng/day (▲) or 500 ng/day (■)] on plasma ACTH (A) and B (B) levels, on rectal temperature (C), body weight change (D), and on food (E) and fluid (F) intake. The i.c.v. cannula was implanted stereotactically 10–11 days before implantation of the osmotic minipumps. Subcutaneous implantation of the pumps and subsequent connection to the i.c.v. cannula was performed between 1430–1630 on day 0 (arrow). Data are expressed as means ± SEM of 10–14 rats. Inset: corrected areas under the curves (AUC) for days 1–3 of i.c.v. infusion of saline (□), 100 ng IL-1β (▲) or 500 ng IL-1β/day (■). ACTH levels of rats infused with 100 ng IL-1β/day are missing due to plasma shortage. * \( P < 0.05 \) vs saline.
Effects of dexamethasone or adrenalectomy (ADX) in rats infused i.p. with IL-1β or saline

Figure 4 shows the effects of treatment of rats with dexamethasone on the earlier mentioned parameters in rats infused with IL-1β (2.0 μg/day) or saline. Daily treatment of rats with dexamethasone completely abolished the effects of IL-1β on plasma ACTH and B levels (ACTH: $P < 0.001$, B: $P < 0.0005$) and on total adrenal weight (57.5 ± 1.5 mg vs 25.1 ± 0.6 mg, saline/IL-1β vs DEX/IL-1β, $P < 0.0005$). The IL-1β-induced decrease in food and fluid intake were significantly reduced by daily dexamethasone treatment (food: $P < 0.01$, fluid: $P < 0.05$).

All of the ADX rats infused with 0.5 μg IL-1β/day had died within 18 h after pump implantation, whereas none of the sham-operated rats died during IL-1β infusion.

DISCUSSION

The present study shows that continuous i.c.v. infusion of low doses of IL-1β in rats stimulates the activity of the HPA axis, increases rectal temperature and decreases food and fluid intake. The results are complementary to those in other recent studies, showing that continuous i.c.v. infusion of IL-1β, in doses as used in this study, decreases the activity of the hypothalamus-pituitary-gonadal axis and decreases thyrotropin-releasing hormone gene expression. It is
of interest that the doses of IL-1β which were effective in the present study (100 and 500 ng IL-1β/day, i.c.v.) were considerably lower than the dose of IL-1β which was needed for stimulation of the pituitary-adrenal axis in the previous study (2000 ng IL-1β/day), in which the cytokine was given by continuous i.p. infusion. These observations suggest that the site of action of IL-1β during chronic i.c.v. infusion is located in the central nervous system.

Pretreatment of animals with the anti-CRH antibody reduced the plasma ACTH response to IL-1β infusion, indicating that the CRH system plays an intermediate role in the stimulation of the HPA axis by chronic i.p. IL-1β administration. This agrees with data of other researchers who did demonstrate such a role for CRH in the IL-1β-induced stimulation of the HPA axis in short-term experiments. It has to be noted, however, that the plasma ACTH response to chronic IL-1β administration was not completely abolished by pretreatment with the anti-CRH antiserum. There are a number of possible explanations for this observation. First, it may be that the amount of the anti-CRH antibody we used was insufficient to fully neutralize all of the secreted CRH. Second, factors structurally distinct from CRH might be involved in the activation of the HPA axis by chronic IL-1β infusion. Chronic stress has been shown to increase vasopressin immunoreactivity in CRH axon terminals in the external zone of the median eminence and acute administration of IL-1β in vivo.

Figure 3. Effects of continuous intraperitoneal (i.p.) administration of saline or 4.0 μg IL-1β/day for 3 days in rats which were treated with saline or with liposomes containing ChMDP, to induce depletion of macrophages [saline/saline (○), saline/IL-1β (▲), ChMDP-liposomes/saline (▲), ChMDP-liposomes/IL-1β ▲], on plasma ACTH (A) and B (B) levels, on rectal temperature (C), body weight change (D), and on food (E) and fluid (F) intake.

ChMDP-liposome suspension or saline was administered intravenously (0.8 ml via the tail vein) and intraperitoneally (0.2 ml) twice (between 0900-1000 and between 1130-1230), 2 days prior to i.p. implantation of the osmotic minipumps (day 0, arrow). Data are expressed as means ± SEM of 5-8 rats.
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Figure 4. Effects of chronic intraperitoneal (i.p.) infusion.

2.0 µg IL-1β/day in rats receiving saline or dexamethasone [SALINE/IL-1β (○), DEX/IL-1β (●)] on plasma ACTH (A) and Infusion of B (B) levels, on rectal temperature (C), body weight change (D), and on food (E) and fluid (F) intake. Saline or dexamethasone (600 µg/injection/rat) was administered subcutaneously twice a day (between 1300-1430 and between 2230-2330) from day −1 up to and including day 2. The osmotic minipumps were implanted on day 0 (arrow). Each point represents the mean ± SEM of 7-8 rats.

has also been shown to induce the release of vasopressin in addition to that of CRH.33,24 Thus, besides CRH, other factors might be involved in the activation of the HPA axis following continuous IL-1β infusion. Third, long term IL-1β administration might directly stimulate the pituitary and/or adrenal gland to release ACTH and B respectively. Indeed, in-vitro IL-1β has been shown to induce ACTH release from mouse anterior pituitary tumor cells25,26 and B release from isolated rat adrenal cells27,28 after a prolonged incubation period (24 h).

To investigate whether IL-1β during chronic administration is also capable of directly stimulating B secretion by the adrenal gland, we infused IL-1β in hypophysectomized rats. However, IL-1β infusion in doses as low as 0.5 µg/day in these rats was lethal within 18 h, most probably because these rats cannot augment pituitary-adrenal activity during IL-1β infusion. This observation underscores that in hypophysectomized rats IL-1β, at least in the first day of infusion, cannot stimulate the adrenal gland directly to a degree sufficient to cope with the stress of chronic IL-1β administration.

It is known that endotoxin and IL-1β can induce the production of IL-1β and tumour necrosis factor-α (TNF-α)29,30 and that macrophages are the main source of endogenous production of these cytokines.31 Therefore, exogenously administered endotoxin or IL-1β might stimulate macrophages to release IL-1β and TNF-α, which in turn could further stimulate the activity of the HPA axis. Derijk and co-workers32 have indeed demonstrated that in rats pretreated with
Cl2MDP-containing liposomes the plasma ACTH and beta response to subpyrogenic doses of endotoxin was completely prevented. Treatment with Cl2MDP-containing liposomes eliminates macrophages as the Cl2MDP-containing liposomes are selectively ingested by macrophages resulting in the death of the macrophages by the toxic effect of Cl2MDP.\textsuperscript{13}

Surprisingly, the effect of IL-1\(\beta\) on the activity of the HPA axis was significantly enhanced instead of decreased in macrophage-depleted rats compared with control rats. It is known that macrophages can also produce cytokines which inhibit the HPA axis, such as interferon-\(\gamma\),\textsuperscript{13} or which can antagonize IL-1\(\beta\) activity, such as IL-1\(\alpha\) receptor antagonist.\textsuperscript{34} It is speculated that decreased production of these peptides accounts for the observed enhancement of the stimulation of the HPA axis by IL-1\(\beta\) in macrophage-depleted rats in our study. In contrast with the additive effect on IL-1\(\beta\)-induced activation of the HPA axis, macrophage depletion had no effect on the increase in rectal temperature and on the anorexia induced by IL-1\(\beta\), implying that macrophage-derived products are probably not involved in these effects. A similar discrepancy was observed in the study using the anti-CRH antiserum: immunoneutralization of CRH decreased the IL-1\(\beta\)-induced activation of the HPA axis whereas it did not significantly alter the effect of i.p. infused IL-1\(\beta\) on rectal temperature and food intake.

The authors investigated whether high or low concentrations of circulating glucocorticoids are capable of modulating the effects induced by chronic i.p. IL-1\(\beta\) infusion. The present study shows that continuous IL-1\(\beta\) infusion, in a dose as low as 0.5 \(\mu\)g/day, in adrenalectomized rats was lethal within 18 h. This observation suggests that an intact function of the adrenal glands is needed to survive the stress induced by IL-1\(\beta\) infusion. The present study also shows that daily administration of high doses of dexamethasone completely abolished the activation of the HPA axis by i.p. infusion of 2.0 \(\mu\)g IL-1\(\beta\)/day. This observation fits well in the concept of immunoregulatory feedback between IL-1\(\beta\) and glucocorticoid hormones, in which glucocorticoids block the production and action of IL-1\(\beta\) thereby protecting the animal from a harmful overshoot of defense reactions.\textsuperscript{3,35}

Infection and inflammation are often accompanied by a sustained activation of the HPA axis and by fever and anorexia. There is compelling evidence that IL-1\(\beta\) is an important trigger for the activation of the HPA axis under these conditions. This study demonstrates that lower doses of IL-1\(\beta\) were able to activate the HPA axis when infused i.c.v. compared with i.p. Furthermore, the present study provides evidence that the CRH system is involved in the activation of the HPA axis by continuous i.p. infusion of IL-1\(\beta\). However, other mediators structurally distinct from CRH, such as vasopressin, might also be involved. This study provides no evidence for a direct stimulatory effect of IL-1\(\beta\) on the release of corticosterone by the adrenal gland which is of sufficient magnitude to resist the stress of chronic i.p. IL-1\(\beta\)-administration. It seems that endogenous macrophage-derived mediators, induced by i.p. IL-1\(\beta\) administration, express an inhibitory rather than a stimulatory effect on the activity of the HPA axis. Finally, exogenous administration of glucocorticosteroids blocks the effect of IL-1\(\beta\) on the HPA axis. Thus, both macrophage-derived products as well as glucocorticoids exert an inhibitory effect on the IL-1\(\beta\)-induced activation of the HPA axis, thereby preventing excessive stimulation of this axis by IL-1\(\beta\), which would result in a dangerous state of suppression of the immune system.

**MATERIALS AND METHODS**

**Materials**

Recombinant human interleukin-1\(\beta\) (IL-1\(\beta\)) was obtained from Glaxo (Glaxo IMB, Geneva, Switzerland). The IL-1\(\beta\) preparation used in experiments 2, 4, and 5 (batch RNB 18511/14-B) has a specific activity of 2.5 \(\times\) 10\(^7\) U/mg protein and the IL-1\(\beta\) preparation used in experiments 1, 3, and 6 (batch RNB 00488/14) has a specific activity of 1.2 \(\times\) 10\(^7\) U/mg protein. According to the specifications of the supplier, endotoxin contamination was negligible (<1.2 ng/mg protein as detected in the limulus amoebocyte lysate assay).

Dexamethasone was obtained from Organon (Oss, The Netherlands). IL-1\(\beta\) was diluted to the desired concentration in pyrogen-free saline just before use. All chemicals used were of analytical grade.

**Animals**

Male Wistar rats (Cpb:WU, 10–12 weeks old, weight 210–230 g in experiments 2–6 and 260–280 g in experiment 1) were obtained from the local breeding facility. The animals were individually housed in Plexiglass cages in an artificially lighted room (lights on at 0700 h; lights off at 1900 h). Rats were provided commercial rat chow (RMH-TM, Hope Farms, Woerden, The Netherlands) and tap water ad libitum. Hypophysectomized and sham-hypophysectomized rats (experiment 3) were provided with 5% glucose in their drinking solution whereas adrenalectomized and sham-adrenalectomized rats (experiment 6) were provided with a 0.9% NaCl drinking solution.

**Experimental procedures**

To diminish the stress by the experimental procedures the animals were handled daily by the experimenter, starting 1 week before cannulation. Blood was collected from conscious, freely moving rats by means of a chronic jugular cannula. Rats were cannulated according to the method described by Steffens\textsuperscript{7} with some modifications as described earlier.\textsuperscript{4} For continuous intracerebroventricular (i.c.v.)
infusion of IL-1β, a stainless steel cannula (Brain Infusion Kit, Alzet Corp., Palo Alto, CA) was stereotactically implanted in the right cerebral ventricle of the brain immediately following jugular cannulation (coordinates according to the Paxinos and Watson atlas: 8.2 mm anterior from the interaural line; 1.4 mm lateral to the antero-posterior suture; 4.5 mm ventral from the surface of the skull). Adrenalectomy (ADX) or sham-ADX was performed using the dorsal approach under halothane (ICI Pharmaceuticals, Macclesfield, UK)–O₂-N₂O anaesthesia between 0930-1130, 4 days after cannulation of the jugular vein. Hypophysectomy (HPX) or sham-HPX was done by the parapharyngeal approach immediately prior to cannulation of the jugular vein.

Four to eleven days after jugular vein cannulation rats were equipped with osmotic minipumps (1.0 μl/h for 3 days, model 1003D, Alzet Corp., Palo Alto, CA), which were loaded with either pyrogen-free saline or saline containing IL-1β. After loading, the pumps were equilibrated, immersed in saline at 37°C for 3-4 h according to the instructions of the manufacturer. The pumps were implanted intraperitoneally (i.p.) under halothane–O₂-N₂O anaesthesia between 1430 and 1630 (experiments 2–6). In experiment 1 the osmotic minipumps were implanted subcutaneously in the neck and connected to the i.c.v. cannula via a polyethylene tube which was also filled with saline or IL-1β solution.

**Protocols**

**Experiment 1**

In this experiment we studied the effects of continuous i.c.v. infusion of saline or IL-1β. The osmotic minipumps were implanted 10–11 days after jugular cannulation and implantation of the i.c.v. cannula. Localization of the i.c.v. cannula was checked at the end of the experiment according to the method of Brakkee et al.² There were three groups: saline, 0.1 μg LL-1β/day and 0.5 μg IL-1β/day.

**Experiment 2**

In this experiment it was studied whether pretreatment of rats with a monoclonal antibody directed against rat CRH (PFU 83, batch 9102-A)² or normal rat IgG (NRI) modulated the effects of continuous i.p. infusion of saline or 4.0 μg IL-1β/day. The anti-CRH antibody or NRI were injected intravenously (i.v.) in a volume of 1.0 ml/rat (100 nmol/ml) 2.5–3 h prior to implantation of the minipumps (day 0), 7 days after jugular cannulation. There were four groups: NRI/saline, NRI/IL-1β, anti-CRH/saline and anti-CRH/IL-1β.

**Experiment 3**

In this experiment the effects of continuous i.p. infusion of 0.5 μg IL-1β/day were examined in HPX or sham-HPX rats. (Sham-)HPX was performed immediately before jugular cannulation, 4 days prior to implantation of the minipumps (day 0). At the end of the experiment rats were decapitated and it was checked macroscopically whether the pituitary gland was completely removed. There were two groups: sham-HPX/IL-1 and HPX/IL-1.

**Experiment 4**

In this experiment the effects of continuous i.p. infusion of saline or 4.0 μg IL-1β/day were studied in macrophage-depleted or control rats. Depletion of splenic, liver and peritoneal macrophages was achieved by injecting rats twice [between 0900–1000 (i.v. and i.p.) and 1130–1230 (i.v. and i.p.)] with liposomes encapsulating dichloromethylene diphosphonate (Cl₃MDP) 2 days prior to implantation of the minipumps (day 0). 0.8 ml of a suspension of Cl₃MDP-containing liposomes was injected i.v. via the tail vein and 0.2 ml of this suspension was injected i.p. Control rats received injections with saline. In this experiment minipumps were implanted 7–8 days after jugular cannulation. There were four groups: saline/saline, saline/IL-1β, Cl₃MDP-liposomes/saline and Cl₃MDP-liposomes/IL-1β.

**Experiment 5**

The effects of continuous i.p. infusion of 2.0 μg IL-1β/day in rats (pre)treated with dexamethasone or saline were investigated. Rats were injected subcutaneously twice a day for 4 days (day –1 up to and including day 2) with dexamethasone (600 μg/0.5 ml) or saline (0.5 ml) between 1300–1430 and 2230–2330. The osmotic minipumps were implanted 7–8 days after jugular cannulation (day 0). There were two groups: saline/IL-1β and dexamethasone/IL-1β.

**Experiment 6**

In this experiment the effects of continuous i.p. infusion of 0.5 μg IL-1β/day were examined in ADX or sham-ADX rats. (Sham-)ADX was performed 4 days prior to implantation of the minipumps (day 0), which were implanted 7–8 days after jugular cannulation. At the end of the experiment rats were decapitated and it was checked macroscopically whether both adrenal glands were completely removed. There were two groups: sham-ADX/IL-1β and ADX/IL-1β.

In all experiments rectal temperature was measured twice a day between 0815-0915 and between 1230-1430 in conscious hand-held rats by insertion of a thermal probe into the rectum. The mean daily rectal temperature for each rat was determined by averaging the morning and afternoon rectal temperatures. Body weight was measured daily at about the same time (0815-0915) and daily body weight change was calculated by subtracting the values measured on subsequent days. Food and water intake was estimated daily between 0815 and 0900 by weighing the residual food pellets and water for individual cages. At the end of the experiments 1–5 (day 3) rats were killed by decapitation, the adrenals removed, freed of fat and weighed. Total adrenal weight is expressed as the summed weight of both adrenals.

In all experiments, blood was collected once a day (between 1000 and 1200) from freely moving rats for 5 days, starting 1 day before implantation of the minipumps (day –1 up to and including day 3). Blood samples (1.8 ml) for measurement of plasma ACTH and B levels, were collected on ice in tubes containing EDTA (45 μl of a 10% (W/V) solution in saline). The samples were gently shaken and spun for 10 min at 1500 × g (4°C). Plasma was separated and red blood cells resuspended in sterile physiological saline (1.3 ml) and returned to each rat. Plasma samples were stored at
- 20°C in aliquots of 250 µl to which 60 KIE aprotinine (Bayer AG, Leverkusen, Germany) was added.

**Hormone measurements**

Plasma ACTH and B values were measured by radioimmunoassay as described by Sweep et al.  

**Statistical analysis**

In the statistical analysis first the areas under the curves (AUC) were calculated. These were corrected for pretreatment values by subtracting the pretreatment value on day 0 from the treatment values on each day (day 1–3). Data of experiment 1 were analysed for a dose-response relationship by using a one-way analysis of variance (ANOVA) of the AUC and the dose of IL-1β. When a significant dose-response relationship was found, Dunnett’s t-test was performed in order to determine which dose of IL-1β induced a significant effect compared with the effect of saline infusion. In the experiments 2 and 4 the AUC’s were analysed by Dunnett’s t-test to determine whether pretreatment of rats with anti-CRH antiserum or ChMDP-liposomes significantly affected basal or IL-1β stimulated plasma ACTH and B levels. Data of experiment 5 (AUCs) were also analysed by Dunnett’s t-test. Differences are considered to be significant if P < 0.05.

**Acknowledgements**

The authors wish to thank Y. Brom, G. Grutters and G. Poelen from the Central Animal Laboratory for expert biotechnical assistance, and P. Somers from the Department of Pharmacology (Organon, Oss, The Netherlands) for performing hypophysectomy. A. Neppelenbroek-Geerts, M. Spruytenburg-van der Wielen, D. Lozekoot and C. Blom-Jongenelen are acknowledged for analytical support. Mr. H. van Lier (Dept. Medical Statistics, Faculty of Medical Sciences, Nijmegen) is acknowledged for statistical advice. This work was supported by the Royal Netherlands Academy of Arts and Sciences.

**REFERENCES**

of the paraventricular nucleus and the median eminence. Neuroendocrinology 57:593–599.


