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Central Administration of IL-1 Reduces Anxiety and Induces Sickness Behaviour in Rats

ALEXANDRA MONTKOWSKI, RAINER LANDGRAF, ALEXANDER YASSOURIDIS, FLORIAN HOLSBOER AND BERND SCHÖBITZ†

Clinical Institute, Max Planck Institute of Psychiatry, 80804 Munich, Germany

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MONTKOWSKI, A., R. LANDGRAF, A. YASSOURIDIS, F. HOLSBOER AND B. SCHÖBITZ. *Central administration of IL-1 reduces anxiety and induces sickness behaviour in rats.* PHARMACOL BIOCHEM BEHAV **58**(2) 329–336, 1997.—In the present study, we examined the effects of various doses of recombinant human interleukin-1β on anxiety-like behaviour, on body temperature, and on behavioural changes typical of sick animals. First, we assessed the behaviour of rats in the elevated plus-maze before and 20 min after intracerebroventricular injection of IL-1 at six doses ranging from 0.001 to 100 ng. After treatment with 0.1 and 100 ng IL-1, animals exhibited different anxiety levels. The dose effect on behavioural performance in the plus-maze appears to be nonlinear (parabolic function), with the highest effects near a 0.1-ng dose and the lowest near doses of 0.0 and 100 ng. In a second set of experiments, we examined the effects of doses of 0.1 and 100 ng IL-1 (which had the most pronounced effects on performance in the plus-maze) on physical parameters over a 24-h period. Using radiotelemetry we measured body temperature, locomotor activity, food intake, and water consumption: a) in animals kept under basal resting conditions, and b) in animals exposed to a novel environment prior to administration of IL-1. Both doses evoked a fever response and reduced locomotor activity, but the increase in body temperature did not correlate with the decrease in locomotor activity and both effects did not occur at the time of behavioural testing. Taken together, our data indicate that central administration of IL-1 has anxiolytic-like properties. © 1997 Elsevier Science Inc.

Elevated plus-maze Interleukin-1 Anxiety Fever Sickness behaviour

EFFECTIVELY combating infectious microorganisms requires rapid activation of immunocytes and cell-to-cell communication, processes that are governed by a variety of soluble factors termed cytokines. The first of these substances to be isolated was interleukin-1 (IL-1), which plays a key role in the initiation and maintenance of inflammatory reactions, lymphocyte stimulation, and the hepatic acute-phase reaction (11). In addition to its peripheral effects, IL-1 acts on the central nervous system (CNS), causing fever and neuroendocrine changes, among which are marked pituitary–adrenocortical activation accompanied by stereotypical behavioural depression including reduced locomotor activity, anorexia, adipsia, and hypersomnia (18,31–33). These central effects are thought to support the host response to infection and to promote recovery from the disease (17,18).

The majority of behavioural experiments using IL-1 have been conducted with rodents and did not include assessment of emotional and affective reactions. However, the presence of IL-1 receptors in neurons of limbic structures and in the cortex of rats and mice (6,32,33) suggests that IL-1 may well affect such reactions. In clinical trials with IL-1, fever and side effects resembling sickness behaviour in animals have been reported in the majority of cancer patients (32,37,38). Moreover, psychiatric symptoms including somnolence, agitation, confusion, and delusions have been observed in a small number of patients treated with IL-1 (37,38). Comparable neurological and psychiatric side effects are known from the administration of other pro-inflammatory cytokines that are marketed drugs, including IL-2 and interferon gamma (12). Furthermore, IL-1 and related cytokines may contribute to the pathology of chronic inflammatory diseases that have CNS involvement, including multiple sclerosis and lupus erythematosus (11,25). Interestingly, patients suffering from these diseases often develop cognitive and affective impairments (21), which raises the question of whether inflammatory mediators can promote or even precipitate these psychiatric disorders during

Requests for reprints should be addressed to Alexandra Montkowski, Department of Neuroendocrinology, Max Planck Institute of Psychiatry, Kraepelinstrasse 2, 80804 Munich, Germany. E-mail: montkow@mpipsykl.mpg.de
†Bernd Schöbitz is deceased.

the course of autoimmune diseases. It is known that the release of IL-1 produces hypercortisolism by activation of the hypothalamic–pituitary–adrenal system, which may affect cognitive and emotional processes (23,35). In agreement with the clinical observation that some patients treated with high doses of cytokines suffer from dysphasia, inability to concentrate, and memory disturbances (12,32) is the finding in an earlier animal study coauthored by one of us (B. Schöbitz) that IL-1 impairs spatial learning in rats (24).

In the present study, we analyzed the effect of various doses of centrally administered IL-1 on the behavioural performance of rats in the elevated plus-maze, a widely used animal test of anxiety (10,16). To determine whether the effects of IL-1 we observed were secondary to fever and sickness behaviour, we also studied the consequences of IL-1 administration on several physical parameters that are typically altered in sick animals (i.e., body temperature, locomotor activity, and food and water intake) by using radiotelemetry and continuous recordings of feeding and drinking. This novel technique allowed both short-term and long-term assessments of locomotor activity in response to centrally administered IL-1. Because exposure of rats to the plus-maze may by itself alter body temperature and locomotor activity, we also measured fever and sickness behaviour in response to IL-1 in animals that received IL-1 under basal resting conditions without novelty stress and in animals that had been exposed to a novel environment in a manner similar to that used in exposures to the elevated plus-maze.

METHODS

Animals and Surgery

Male adult Wistar rats (180–220 g), purchased from Charles River (Sulzfeld, Germany), were housed five per cage with free access to standard chow and drinking water under conditions of constant temperature (21 ± 2°C) and a 12 L:12 D cycle (lights on at 0800 h). Polyethylene guide cannulas (internal diameter 0.4 mm, external diameter 0.8 mm) were implanted into the right lateral cerebral ventricle under halothane (Hoechst, Frankfurt, Germany) anaesthesia according to a method described previously (5). The position of the cannula relative to bregma was lateral 0.9, ventral −1.5, and 3.2 mm below the surface of the skull, according to the atlas of Paxinos and Watson (26). After surgery, the rats were housed singly until the experiment started, i.e., for 5–8 days. At the end of each experiment, the animals were killed with an overdose of halothane. The experiments were approved by the Committee on Animal Care and Use of the relevant local governmental body.

Drug Administration

Recombinant human IL-1β was a generous gift from Dr. S. Gillis, Immunex (Seattle, WA, USA). The specific biological activity was 1×10^9 units/mg in the thymocyte proliferation assay. IL-1β was dissolved in 0.9% saline, and the final concentration of the solution was adjusted to give a total volume of 5 μl per dose. The solution was injected intracerebroventricularly (ICV) into freely moving animals over a period of approximately 2 min via a polyethylene tube connected to the injection needle and the syringe.

Behavioural Testing in the Elevated Plus-Maze

One day before the experiment, animals were moved from the housing colony to the testing room to enable adaptation to the new environment. The elevated plus-maze is a symmetrical cross made of grey plastic and consisting of two opposing arms that are open to the environment (open arms) (50 × 10 cm), two arms that are enclosed by side and end walls (closed arms) (50 × 10 × 40 cm), and an open central area (10 × 10 cm) that connects all four arms. The maze is elevated to a height of 75 cm from the floor. At the start of the experiment, animals were placed into the central area of the maze facing a closed arm. Behavioural testing was recorded via a video camera. An observer who was unaware of the treatment, measured open- and closed-arm entries and the amount of time the animals spent in each type of arm during a 5-min exposure. The ratio of time spent in the open arms to total time spent in both types of arms is considered to be a measure of a rat's level of anxiety. The higher the ratio, the less anxious the animal. General activity is expressed as the number of closed-arm entries. An entry was defined as all four paws in one arm. In the first experimental series, there were seven conditions. In each condition, six to eight rats were exposed to the maze twice, once immediately before and once 20 min after administration of IL-1 or vehicle [six doses of IL-1 (0.001, 0.01, 0.1, 1, 10, and 100 ng) were tested and compared with vehicle (5 μl saline)].

Measurement of Body Temperature, Locomotor Activity, Food Intake, and Water Consumption

In a second set of experiments, performed after evaluation of the dose effect of IL-1 on behavioural performance in the plus-maze, we determined body temperature, locomotor activity, food intake, and water consumption in rats that received in a randomised order either saline or one of the two most effective doses of IL-1 (0.1 and 100 ng). Because novel environmental stress such as exposure to the plus-maze prior to administration of a substance may alter behavioural and physiological parameters, the animals were injected with vehicle and IL-1 under two conditions: a) basal resting conditions, i.e., after a 2-day adaptation period in the telemetry cages, and b) after exposure to a novel environment. To assess physiological parameters under novelty stress conditions, rats were put into the telemetry cages for about 5 min. They were then returned to their home cages, where they received 5 μl of saline or 0.1 or 100 ng IL-1 (again, randomized choice assignment). After 20 min, the rats were reexposed to the telemetry cages and radiotelemetric measurements were made for 24 h. In randomisation, particular care was taken that the groups of rats were of similar size (each group had to contain six to eight animals).

Core body temperature (in °C) and locomotor activity (expressed in arbitrary units) were monitored in undisturbed rats with a radiotelemetric method using the Dataquest IV system (Data Sciences International, St. Paul, MN, USA). A battery-powered transmitter was implanted into the peritoneal cavity of each animal under halothane anaesthesia. After surgery, animals were allowed to recover for 2 days. The frequency of the signal emitted by the transmitter is proportional to the animal's body temperature. The signal was first transmitted to a receiver beneath the cage and from there it was transferred to and processed by an IBM personal computer. Body temperature was recorded at 5-min intervals over a 24-h period.

Locomotor activity was measured by monitoring changes in the strength of the signal received from the transmitter. Changes in signal strength generated a digital pulse, which was counted by the Dataquest IV system. At a rate of motion of >1 cm/s, the number of pulses depended strictly on the dis-

tance the animal moved. Locomotor activity was recorded at 5-min intervals over a 24-h period.

Food intake was determined by calculating the difference between the weight of the food pellets in the animal cages before injection and 12 h postinjection and between 12 and 24 h postinjection.

Water consumption was determined by monitoring the licking rate (expressed in licks/5 min) with lick sensors (Data Sciences International) connected to the drinking nipple and to the ground wire attached to the cage. Touching the nipple during water intake generated a pulse that was received by the Dataquest IV system. As with body temperature and locomotor activity, licking rate was recorded at 5-min intervals over 24-h period.

Statistics

Results are expressed as means ± SEM. For statistical evaluation of behavioural performance reflected by the variables ratio time (RT) and general activity (GA), a two-factorial multivariate analysis of variance (MANOVA) with repeated-measures design was performed. Dose was a between-subjects factor (with seven levels) and treatment a within-subjects factor (with two levels: pre- and postinjection). Prior to MANOVA, the variables RT and GA were transformed (RT with a sine transformation and GA with a square root transformation) to try to approach normality and homogeneity of the data. Additionally, to fit a curve to the dose–effect data, a trend analysis (tests with polynomial contrasts) within the analysis of variance (ANOVA) was performed.

Effects of saline and of 0.1- and 100-ng IL-1 doses on body temperature and locomotor activity over a 24-h period in the two experimental conditions were tested for significance by a three-factorial MANOVA with repeated-measures design. In this case, dose and condition were between-subjects factors (with three and two levels, respectively) and time a within-subjects factor. For statistical reasons (avoidance of colinearities and singularities), the time effect (duration of efficacy of IL-1) was not evaluated over the 5-min intervals, but over four 6-h intervals, each being one-fourth of the 24-h period. Two time series indicators, the mean location (ML) and the area under the curve (AUC), calculated for each of the 6-h intervals were then used in the MANOVA. For food intake and licking rate, a three-factorial ANOVA with repeated-measures design was also performed, but the within-subjects factor time was considered to have only two levels (the intervals 0–12 and 12–24 h postinjection). If there were significant main or interaction effects in the MANOVAs, univariate *F*-tests were performed to identify those variables that contributed significantly to the factor effects. For each of these variables, the Scheffé tests and tests with contrasts were then performed to locate the levels of the between- and within-subjects factors, respectively, that differed significantly among the variables. As the nominal level of significance for testing the null hypothesis of factor effects, $\alpha = 0.05$ was accepted. For post hoc comparisons, a reduced level of significance (adjustment according to the Bonferroni procedure) was used to keep the type I error ≤ 0.05.

RESULTS

Effect of IL-1 on Behavioural Performance in the Elevated Plus-Maze

Increasing doses of IL-1 yielded an inverted-U shaped dose–effect curve for the relationship between the IL-1 dose and the behavioural response (Fig. 1). ANOVA revealed significant main and interaction effects of dose and treatment on the animal's behavioural performance [effect of dose: $F(12, 70) = 3.40$, $p = 0.001$; effect of treatment: $F(2, 35) = 4.48$, $p = 0.026$; effect of dose × treatment: approx. $F(12, 70) = 2.08$, $p = 0.037$; Wilks multivariate tests of significance]. Whereas both main effects significantly influenced only RT [dose effect on RT: $F(6, 36) = 5.23$, $p = 0.001$; treatment effect on RT: $F(2, 35) = 3.52$, $p = 0.045$; univariate *F*-tests in MANOVA], the dose × treatment interaction affected GA as well as RT [for both parameters: minimum $F(6, 36) = 2.58$, $p < 0.042$]. Having found an interaction effect, we then investigated the effect of the various doses in each level of the treatment factor and vice versa. The means of RT and GA before and after injection with various doses of IL-1 are shown in Fig. 1 and Table 1, respectively. Saline-treated animals and animals treated with 0.001 ng IL-1 showed higher RT values before than after treatment, although the differences were not significant. However, at higher IL-1 doses the effect was reversed: RT values after injection of more than 0.1 ng IL-1 were higher than before treatment. As with the very low doses, the very high dose of 100 ng caused a reduction in RT values compared with pretreatment. The only IL-1 doses with significantly different RT values before and after treatment were 0.1 and 100 ng. Compared with preinjection values (tests with contrasts) and values for saline-treated animals (Scheffé tests), rats receiving 0.1 or 100 ng of IL-1 spent significantly more (0.1-ng dose) and less (100-ng dose) time, respectively, in the open arms of the plus-maze (*p*-values < 0.05). GA was not significantly altered in the groups treated with doses below 100 ng of IL-1 compared with saline-treated controls. The GA values did not show significant differences between pre- and postinjection for any dose except the 100-ng dose (*p*-value for the 100-ng dose < 0.05; tests with contrasts). In those treated with 100 ng of IL-1, GA was significantly reduced, by over 81% (*p*-values < 0.05; Scheffé tests). Partitioning of the between-dose sums of RT into trend components (linear, quadratic, cubic,

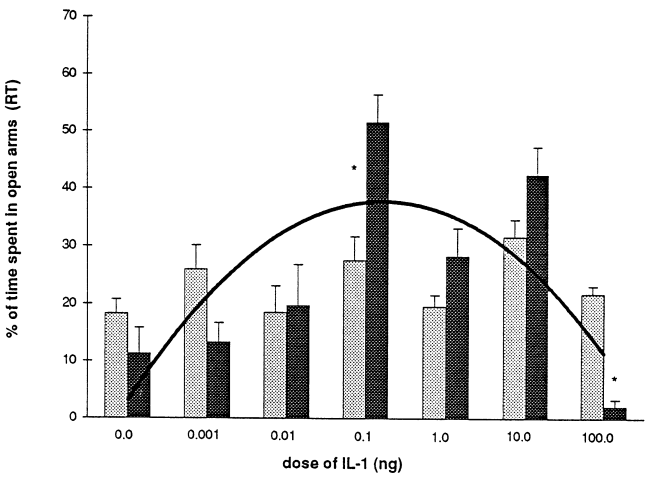

FIG. 1. Percentage of time spent in open arms (RT) of the elevated plus-maze before and 20 min after ICV injection of saline and various doses of IL-1. The dose effect on RT follows a quadratic trend (second-degree polynomial fit). Lightly shaded bars, saline; heavily shaded bars, IL-1; curve, dose fit; RT, ratio time, i.e., percentage of time spent in open arms vs. time spent in both types of arms. *Significant difference (Scheffé test or test with contrasts in ANOVA; see text).

TABLE 1

GENERAL ACTIVITY IN THE PLUS-MAZE BEFORE AND 20 MIN AFTER ADMINISTRATION OF IL-1 OR SALINE

IL-1		General Activity (mean ± SE)		
Level Number of Dose Factor	Dose (ng)	Before Injection	After Injection	Outcomes: Treatment
0	0.000	7.0 ± 1.2	6.2 ± 2.2	NS
1	0.001	7.5 ± 0.8	7.0 ± 1.1	NS
2	0.010	7.2 ± 1.0	6.7 ± 0.7	NS
3	0.100	6.8 ± 0.8	7.1 ± 0.6	NS
4	1.000	7.3 ± 0.9	7.1 ± 1.0	NS
5	10.000	7.8 ± 1.5	9.0 ± 0.4	NS
6	100.000	7.9 ± 0.9	1.2 ± 0.2	*

	Before Injection						After Injection					
	0/1	0/2	0/3	0/4	0/5	0/6	0/1	0/2	0/3	0/4	0/5	0/6
Outcomes: Dose	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*

"Outcomes: Treatment" refers to test outcomes for general activity differences before and after treatment (tests with contrasts). "Outcomes: Dose" refers to test outcomes for general activity differences between doses (Scheffé test); 0/1, 0/2, etc. refer to dose level 0 vs. dose level 1, dose level 2, etc. *$p < 0.05$; NS, not significant.

etc.) and testing of their fit to the dose group means with polynomial contrasts in ANOVA indicates that a second-degree polynomial appears to fit the dose–effect data best [effect of the quadratic trend component: $F(1, 36) = 20.99$, $p = 0.0001$ (Fig. 1)].

Effect of IL-1 on Body Temperature and Locomotor Activity

Figures 2a and 3a show the hourly mean values of body temperature curves over 24 h for control rats (saline injection) and for rats injected with 0.1 or 100 ng IL-1 under basal resting conditions and novelty stress. Figures 2b and 3b show mean locomotor activity in the four 6-h intervals under the two conditions. Two ANOVAs, one with the AUC and one with the ML values of body temperature and locomotor activity, revealed in both parameters significant main effects for the factors dose and time as well as significant dose × time and condition × time interactions [effect of dose on AUC and ML: minimum $F(4, 62) = 9.50$, $p < 0.0001$; effect of time: minimum $F(6, 27) = 5.61$, $p < 0.001$; effect of dose × time: minimum $F(12, 190) = 1.81$, $p < 0.048$; Wilks multivariate tests of significance]. Both body temperature and locomotor activity contribute significantly to these effects ($p < 0.05$ for body temperature and locomotor activity for each of the factors; univariate *F*-test in MANOVA). Examination of the dose effect on body temperature showed that both doses of IL-1 (0.1 and 100 ng) were pyrogenic in both experimental conditions. In the first 6 h, the body temperature of IL-1-treated animals increased continuously, which led to significantly higher AUC and ML values than in the controls for this interval (p-values < 0.05 for both AUC and ML values; Scheffé tests). The 100-ng IL-1 dose evoked a prolonged fever response that lasted 2–20 h postinjection (Figs. 2a, 3a). This fever response was significantly higher than that of the control rats for each experimental condition and for all time periods except the last one (19–24 h) (p-values < 0.05; Scheffé tests). For the second and third 6-h intervals, rats treated with 0.1 ng IL-1 under novelty stress also differed significantly in body temperature from those treated with 100 ng IL-1 (p-values < 0.05; Scheffé tests). Pairwise comparisons of the various time intervals yielded significant differences in mean body temperature between the first (0–6 h) and last (18–24 h) 6-h intervals in the basal condition for the 100-ng IL-1 dose and between the first (0–6 h) and each of the following 6 h intervals (i.e., 6–12 h and 12–18 h) in the novelty stress condition for 0.1 ng IL-1 (p-values < 0.05; tests with contrasts).

Examination of the dose effect on locomotor activity in animals monitored under basal resting conditions (Fig. 2b) showed that locomotor activity was lower in the animals injected with 0.1 or 100 ng IL-1 than in the control rats. Locomotor activity increased with time, but it remained significantly lower than that of the control rats (p-values < 0.05; Scheffé tests). During the two last time intervals, the 100-ng dose of IL-1 reduced locomotor activity more than the 0.1-ng dose of IL-1. The suppression of locomotor activity in rats tested under novelty stress conditions (Fig. 3b) showed a time course and values similar to those seen in animals kept under resting conditions. However, in contrast to the effect in unstressed rats, rats that were injected with 0.1 ng IL-1 under novelty stress conditions did not show significantly lower locomotor activity than the controls. Nineteen hours after exposure to the telemetry cages, the locomotor activity of the IL-1-treated animals had approached the levels of the control animals.

Body Temperature and Locomotor Activity During the Test Interval on the Plus-Maze

To assess the efficacy of IL-1 immediately after injection, we investigated the animal's body temperature and locomotor activity every 10 min during the first hour after substance administration for the novelty stress condition (Fig. 4). ANOVA with the mean values, time being a within-subjects factor with six levels, revealed a significant main effect for dose and time as well as a significant dose × time interaction effect [effect of dose: $F(4, 32) = 3.65$, $p = 0.015$; effect of time: $F(10, 8) = 5.95$, $p = 0.009$; effect of dose × time: $F(20, 16) = 2.53$, $p = 0.040$; Wilks multivariate tests of significance]. The dose and time effect were most pronounced in locomotor activity measures, which varied reasonably between the various time points and doses.

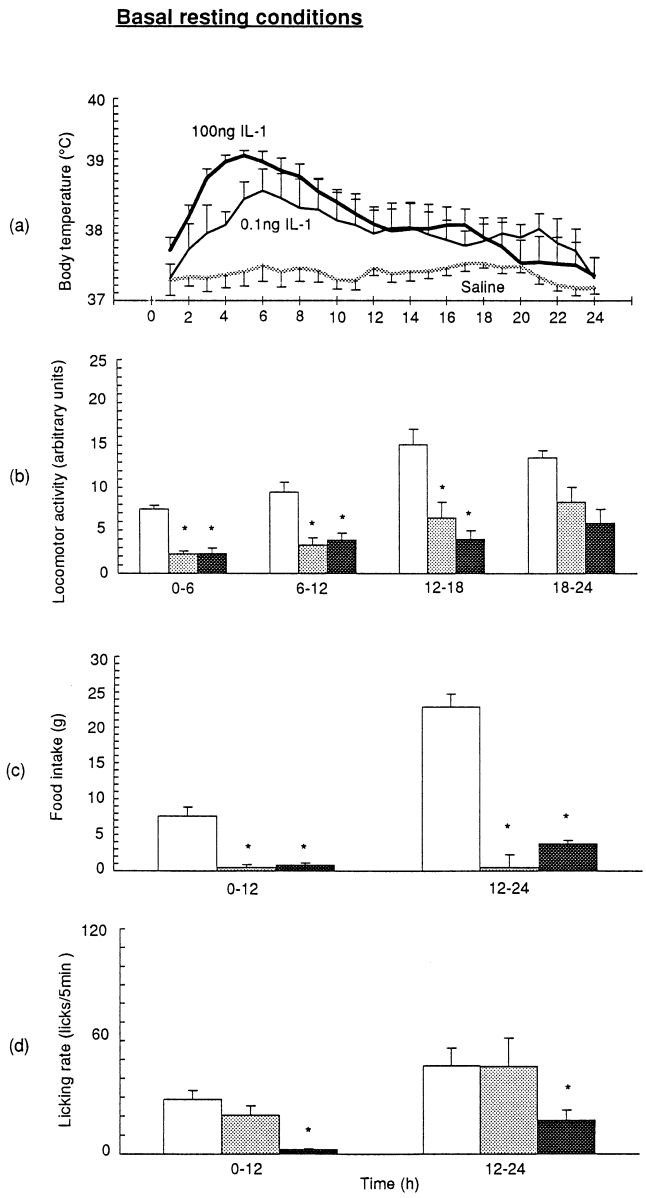

FIG. 2. Effect of different doses of IL-1 on four measures of physical performance over a 24-h period under basal resting conditions. Course of body temperature over 24 h (a); mean locomotor activity over four consecutive intervals (6 h each) (b); and food intake (c) and licking rate (d) over two consecutive time intervals (12 h each) after substance administration. Empty bars, saline; lightly shaded bars, 0.1 ng IL-1; heavily shaded bars, 100 ng IL-1. *Significant differences (Scheffé test or test with contrasts in ANOVA; see text).

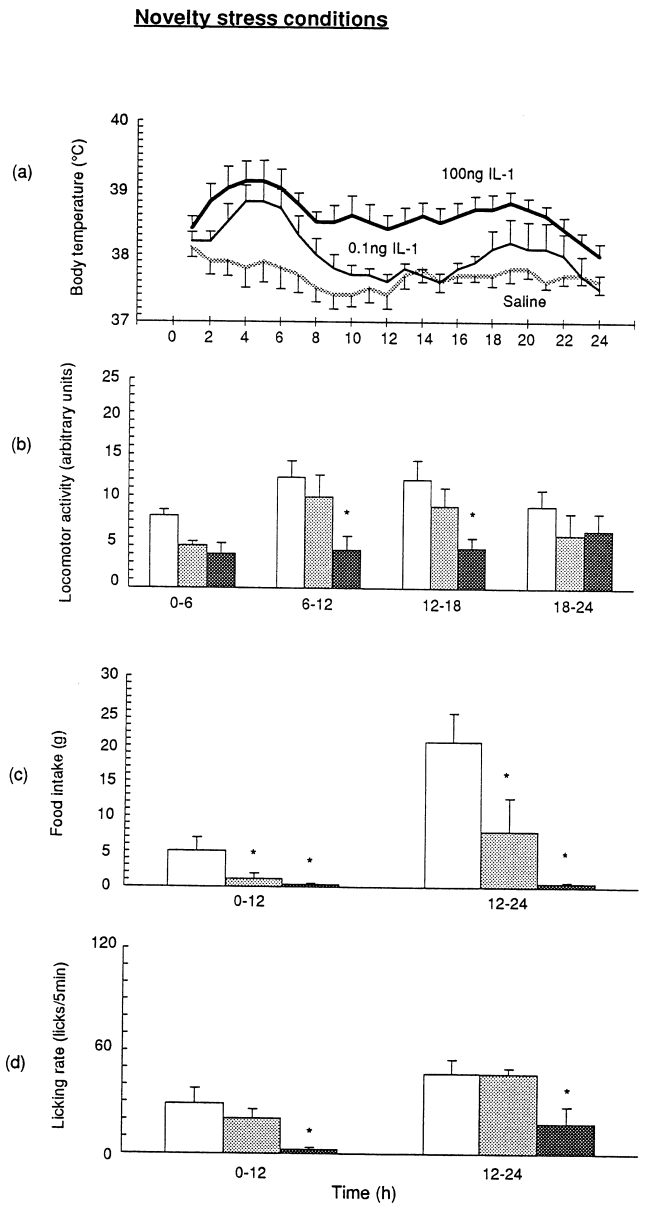

FIG. 3. Effect of different doses of IL-1 on four measures of physical performance over a 24-h period under novelty stress conditions. Course of body temperature over 24 h (a); mean locomotor activity over four consecutive intervals (6 h each) (b); and food intake (c) and licking rate (d) over two consecutive time intervals (12 h each) after substance administration. Empty bars, saline; lightly shaded bars, 0.1 ng IL-1; heavily shaded bars, 100 ng IL-1. *Significant differences (Scheffé test or test with contrasts in ANOVA; see text).

Although IL-1 injection induced an increase in body temperature, and the saline- and 0.1-ng IL-1-treated rats started to explore the cages, thereby increasing their level of locomotor activity, there was no significant difference between these groups in body temperature and locomotor activity for the first four 10-min intervals (Fig. 4a, b). Locomotor activity in rats that received 100 ng IL-1 was significantly lower than in those treated with saline in the first two and in the last 10-min intervals (p-values < 0.05; Scheffé tests). In the novel environment, rats treated with either saline or 0.1 ng IL-1 had an approximately 2.5-fold higher level of locomotor activity during the first 10 min than did rats injected with 100 ng IL-1. Ten minutes after the beginning of the novelty stress, the saline- and 0.1-ng IL-1-injected animals reduced investigation of the cages, and their locomotor activity (Fig. 4b) approached basal resting condition levels (data not shown in the figure) and did not differ significantly for any of the consecutive 10-min intervals. Body temperature gradually decreased after the novelty

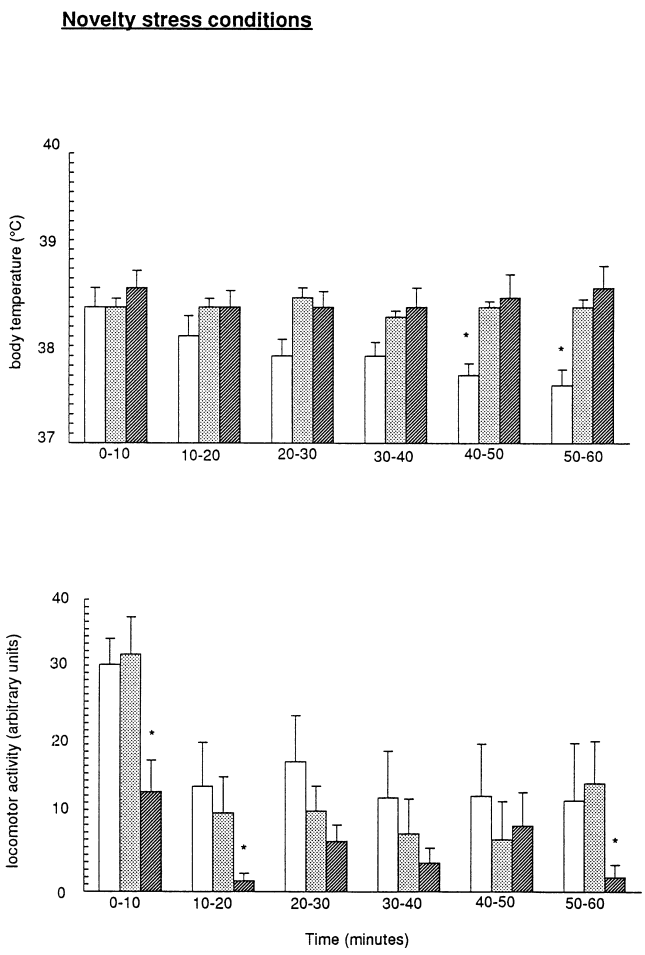

FIG. 4. Mean levels of body temperature (a) and locomotor activity (b) in consecutive 10-min intervals during the first hour after substance injection in rats kept under novelty stress conditions. Empty bars, saline; lightly shaded bars, 0.1 ng IL-1; heavily shaded bars, 100 ng IL-1. *Significant differences (Scheffé tests or tests with contrasts in ANOVA; see text).

stress in the saline-treated controls, but not in the animals treated with the two doses of IL-1. Significant differences in body temperature between saline- and IL-1-treated animals (Fig. 4a) occurred 40–60 min postinjection (p-values < 0.05; Scheffé tests).

Effect of IL-1 on Food Intake and Water Consumption

Figures 2c and d and 3c and d show the mean values for food intake and water consumption (licking rate) for the two 12-h intervals under basal resting conditions (no novelty stress) and novelty stress, respectively. Both parameters were significantly influenced by the dose, time, and dose × time factors [dose effect: $F(4, 58) = 16.50$, $p < 0.0001$; time effect: $F(2, 29) = 28.95$, $p < 0.0001$; dose × time effect: $F(4,58) = 6.65$, $p < 0.0001$; ANOVA with repeated-measures design and Wilks multivariate tests of significance]. Independent of the experimental condition, both IL-1 doses used caused a significant reduction in food intake during both 12-h intervals compared with saline, whereas only the 100-ng IL-1 dose reduced the licking rate significantly (Scheffé tests, p-values < 0.05). Comparison of mean food intake and licking rates for the first and second 12-h intervals for all doses and conditions revealed a significant difference only for food intake after saline treatment (p-values < 0.05; tests with contrasts).

DISCUSSION

Our results demonstrate that IL-1 administration into the lateral ventricle of the rat brain has different effects on behavioural performance in the elevated plus-maze depending on the dose and the experimental design used. A low dose of IL-1 (0.1 ng) increased open-arm exploration in rats that had explored the maze once before they were injected, indicating an anxiolytic-like effect of IL-1. Conversely, a high dose of IL-1 (100 ng) reduced exploration of both open arms and closed arms. To sort out whether these effects do in fact reflect an altered anxiety level or merely reduced locomotor activity, we conducted a second set of experiments. We found that both the low dose and the high dose induced sickness behaviour, as shown by a novel method for assessing this type of behavioural depression. However, only the high dose altered behavioural performance in the plus-maze, via its depression of locomotor activity. Therefore, we were unable to differentiate clearly between an increased anxiety level and a false-negative effect due to reduced locomotion.

Behavioural performance in the elevated plus-maze is governed by both the exploratory drive of laboratory rodents toward the novel environment and the avoidance drive caused by the innate fear of open spaces (22). This test allows detection of anxiety-like behaviour and assessment of the general activity of animals in the same experimental session and is thus widely used to investigate putative anxiolytic and anxiogenic drugs (10,16). We tested the animals before and 20 min after injection because this design allowed us to compare intraindividual differences in the behavioural performance of IL-1-treated animals and controls. With this experimental design we previously found the low dose of IL-1 to have an anxiolytic effect in animals exposed to the plus-maze once before treatment, but no such effect could be observed in those with no previous plus-maze experience (data not shown). Therefore, we assume that the observed effect is triggered by the prior maze experience, which reinforces the level of anxiety (14) and may be mandatory for the anxiolytic-like effect of IL-1 to emerge. This view is supported by a study by File (13) showing that handling and prior maze experience modify behavioural and neurochemical effects of anxiolytic drugs. In contrast, other investigators (9,39) found that repeated exposure to the plus-maze can lead to habituation and thus to a decreased level of anxiety. In the present study we observed a distinct decrease in time spent in the open arms during the second exposure in animals treated with low doses of IL-1 and in controls; this decrease was not accompanied by a change in the level of general activity. We therefore conclude that reexposure to the maze does in fact enhance anxiety and that this effect is inhibited by IL-1.

Discrepancies in the behavioural performance of animals reexposed to the plus-maze according to different protocols are probably due to the rat strains used, the clock time of testing the animals, and the interval between the first and second exposure. Another possible explanation for our data is that IL-1 administration mimics an anxiolytic-like effect due to enhanced locomotor activity. However, both radiotelemetric measurements and determination of the number of closed-

arm entries rule out this possibility. These analyses also suggested that the effect of 100 ng IL-1 on RT reflects immobility caused by the induction of sickness behaviour rather than anxiety.

Radiotelemetric measurements also showed that there was no difference in body temperature in vehicle- and IL-1-treated animals after the second exposure to the plus-maze (Fig. 4a), which excludes an effect of body temperature on the anxiolytic-like effect of IL-1. In agreement with our finding using radiotelemetry, Plata-Salaman (27) and Bianchi et al. (3) also found that centrally administered IL-1 suppresses spontaneous locomotor activity. However, rats treated with 0.1 ng IL-1 20 min before exposure to the plus-maze did not differ from controls in their locomotor activity, as evidenced both by radiotelemetry and by determination of the number of closed-arm entries as a measure of general activity in the plus-maze.

Because IL-1 is thought to be a principal endogenous mediator of fever and sickness behaviour (11,17,18,31), we investigated the influence of the most effective doses of this cytokine (0.1 and 100 ng) on body temperature and sickness behaviour in view of a possible contribution of these autonomic and behavioural reactions to performance in the plus-maze. Thus far, various animal models have been used to quantify sickness behaviour, including: a) conditioned taste aversion (15), b) social exploration (investigation of a juvenile by an adult conspecific rodent) (7), and c) schedule-controlled behaviour (food-rewarded lever-pressing on a fixed ratio 10) (8). These tests are based on determination of spontaneous or acquired ongoing behaviour, which is suppressed in sick animals. In contrast to these paradigms, we measured sickness behaviour in rats under basal resting conditions and after exposure to a novel environment by using radiotelemetric quantification of body temperature and locomotor activity in combination with continuous recordings of food intake and water consumption. This technique allows analysis of sickness behaviour in response to various pro-inflammatory stimuli such as lipopolysaccharides, IL-6, and nucleic acids (G. Pezeski and B. Schöbitz, unpublished observations) and can be used for pharmacological screening of drugs that can inhibit sickness behaviour.

The radiotelemetric data obtained after the animals had either been exposed to a novel environment situation (non-stressed condition) or not (stressed condition) suggest the following. a) First, among the parameters we analyzed, anorexia seems to be the most sensitive reaction of animals in response to pyrogenic and pro-inflammatory agents, and it can occur in the absence of reduced locomotor activity (Figs. 2b, c, 3c, b). b) Second, adipsia is less likely to develop in sick animals than the other symptoms we measured. In our experiments, reduced water consumption was observed only if a large amount of IL-1 (100 ng) was given. This is in line with data from other investigators (27,28), who found that IL-1 has stronger effects on food intake than on water consumption. Apparently IL-1 administration dissociates food and water intake, which are normally closely interrelated via prandial drinking. Finally, in sick animals there is no correlation between the increase in body temperature and locomotor activity (Figs. 2a, b, 3a, b). The difference between the parameters was most prominent 15–18 h after administration of 0.1 ng IL-1, when locomotor activity was markedly depressed in the absence of fever. Apparently, fever or elevated body temperature per se does not produce sickness behaviour. Accordingly, fever and behavioural alterations that accompany infectious diseases can be separated under certain experimental conditions [(18) and references therein]. For example, corticotropin-releasing hormone (CRH) antagonists attenuate IL-1-evoked fever but not food-motivated behaviour (4,30).

Both central and peripheral administrations of IL-1 produce changes in neurotransmission in limbic structures and other brain areas that may be involved in the anxiolytic-like effect we observed in this study. For example, local administration of IL-1 into the hippocampus and hypothalamus increased the extracellular concentration of 5-hydroxytryptamine (5-HT) in the respective brain area (19,36), which points to an involvement of 5-HT in centrally mediated effects of IL-1. In fact, 5-HT seems to affect anxiety via 5-HT_1A and 5-HT_2 receptors (1). Electrophysiological effects of IL-1 are also compatible with our findings because IL-1 enhances synaptic inhibition in hippocampal CA1 neurons (42), which is thought to be a $GABA_A$-ergic effect of this cytokine. Accordingly, IL-1 augments $GABA_A$-dependent chloride uptake in murine synaptoneurosomes and enhances the $GABA_A$-mediated increase in chloride permeability in chick cortical neurons (20). In addition, IL-1 elevates the threshold dose for pentylenetetrazol-induced seizures in mice, which is typical for $GABA_A$-ergic anxiolytic drugs (20).

IL-1 stimulates CRH secretion from hypothalamic parvocellular neurons into the portal vein system (2). If this process is accompanied by intracerebral release of CRH, as has been described recently in the amygdala in response to restraint stress and ethanol withdrawal, one might expect an anxiogenic rather than an anxiolytic effect of IL-1. Our data suggest that either this is not the case, at least under our experimental conditions, or the activity of CRH is compensated by other factors that affect anxiety, such as neuropeptide Y.

Further studies are needed to evaluate whether the anxiolytic-like effect of IL-1 is also detectable in other animal tests of anxiety and whether this effect has any physiological significance. Because behavioural changes caused by IL-1 and other cytokines are considered to be adaptive processes that serve to optimize host defense mechanisms (17,18,29), anxiolysis may be a component of an immediate response to infection, causing the animal to relax and thereby contributing to increased sleep duration and recovery from the infection. In addition, IL-1 may counterregulate the effects of other mediators that are released in sick animals and that would prevent the induction of sleep and sickness behaviour (32,34). In view of the putative involvement of IL-1 in the pathology of chronic inflammatory and autoimmune diseases such as multiple sclerosis and lupus erythematosus (11,25), IL-1 and related cytokines may contribute to precipitating affective disorders in patients with these diseases, including paradoxical symptoms such as euphoria, agitation, and mania (21). Recently, IL-1 has even been proposed to be involved in the pathogenesis of psychiatric disorders, including major depression (40) and schizophrenia (41). In this context it is desirable to acquire further data on emotional and cognitive alterations caused by IL-1, because this information might contribute to our understanding of disturbances of affect and memory in patients suffering from inflammatory diseases with CNS involvement and of paradoxical central side effects in response to IL-1 (37,38).

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