The Effects of Psychological Stress on Plasma Interleukin-6 Activity in Rats

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LÉMAY, L. G., A. J. VANDER AND M. J. KLUGER. The effects of psychological stress on plasma interleukin-6 activity in rats. PHYSIOL BEHAV 47(5) 957-961, 1990. — The purpose of this study was to determine the effects of a particular psychological stress, exposure to an open-field, on plasma IL-6 activity in rats. Plasma IL-6 activity was 40.6 ± 7.2 units/ml in control rats, 105 ± 6.8 units/ml after 30 minutes exposure to an open-field, and 221 ± 17 units/ml after 60 minutes of exposure (p = 0.0003). There was a positive correlation (r = 0.71, p = 0.043) between the change in plasma IL-6 activity and body temperature. However, we conclude, based on earlier data relating plasma IL-6 activity to body temperature changes following injection of lipopolysaccharide, that the plasma levels of IL-6 following exposure to an open-field are not high enough to account for the rise in body temperature observed in rats during this stress. In conclusion, these experiments indicate that exposure to psychological stress can elevate the plasma concentration of IL-6, a known mediator of the acute phase response.

Stress hyperthermia Body temperature Cytokines Anesthesia Methoxyflurane Ketamine hydrochloride Xylazine

ONE response to certain psychological stresses, which is common both to animals and humans, is an elevation in body temperature termed “stress-induced hyperthermia” (2-4, 11, 15, 18). A portion of this hyperthermia can be blocked by cyclooxygenase inhibitors such as sodium salicylate and indomethacin (4, 11, 18). These data support the hypothesis that part of the stress-induced hyperthermia is mediated by prostaglandins in the central nervous system (CNS) (20). It is currently thought that during fever, cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) are responsible for the rise in central nervous system prostaglandins (7,20). Recently, interleukin-6 (IL-6), a cytokine that is thought to be responsible for part of the acute phase response (9, 10, 14, 16, 22), has also been found to be capable of producing fevers (10), and we have found that IL-6-induced fevers are blocked by indomethacin (12). This raises the possibility that stress-induced hyperthermia may be caused by increased release of IL-6.

The major hypothesis we have tested in this study is that exposure of rats to open-field stress results in an elevation in plasma IL-6 levels. We were also interested in whether the rise in body temperature and the observed increase of plasma IL-6 activity in response to open-field stress showed adaptation after multiple exposures to the open-field. Another hypothesis that was tested was that the stress of exposure to anesthetics would result in a rapid rise in plasma levels of IL-6.

METHOD

Animals

Forty-six specific pathogen-free male Sprague-Dawley rats weighing 180–220 g were obtained from Charles River (Portage, MI). Rats were housed at 23–25°C with a 12/12 hr light-dark cycle and given ad lib tap water and rodent chow. Rats were individually housed for about 2 weeks before the experiment.

Measurement of Body Temperature

Core temperature was measured by biotelemetry using transmitters implanted intraperitoneally (IP) (Mini Mitter, Inc., Sunriver, OR) (17). The transmitters were implanted at least 4 days before experiments. Each transmitter was calibrated prior to implantation. Output (frequency in Hz) was monitored by a mounted antenna placed under each rat’s cage and fed into a peripheral processor (Dataquest III system, Mini Mitter, Inc.) connected to an IBM-PC. Temperatures were recorded at 1-minute intervals.

IL-6 Bioassay

IL-6 activity in plasma was measured using the IL-6-dependent B-9 hybridoma cell line kindly provided by Dr. Lucien Aarden (Central Laboratory of the Netherlands) (1,21). The B-9 cells were cultured in Iscove’s modified Dulbecco’s medium (IMDM; Life Technology, Inc.) supplemented with human recombinant IL-6 (8 units/ml obtained from L. Aarden), 20 μM 2-mercaptoethanol, 5% heat-inactivated fetal calf serum (FCS), 100 IU/ml penicillin and 100 μg/ml streptomycin. Cells were washed once in the above medium without added IL-6 before the addition of the plasma samples or known amounts of human rIL-6, also provided by Dr. Aarden.

To run the IL-6 assay, 100 μl of 1:10 diluted plasma sample was combined with 5000 B-9 cells in 100 μl IMDM/5% FCS in flat-bottom microtiter plates (Corning) for a final volume of 200 μl. All samples were run in duplicate. The control medium, which
exposed to the open-field for 30 minutes per day for the first seven
weeks. The B-9 assay was run as described above except that 2 µg
of antibody against mouse IL-6 was added to the serially diluted
plasma sample and incubated for 1 hour before addition of the B-9
cells.

For the serially diluted samples, all counts per minute (cpm)
that fell within 2 standard deviations of the baseline cpm (i.e.,
within the 95% confidence interval) were excluded to reduce the
potential error resulting when converting from cpm to units of IL-6
by multiplying by the dilution factor. The largest calculated IL-6
activity that fell outside the 95% confidence intervals was taken as
the IL-6 value; this ensured that all data used fell in the steep linear
portion of the standard curve.

To determine that plasma IL-6-like activity in the rat plasma
was really due to IL-6, we measured this activity in the B-9 assay
after addition of rabbit antibody to mouse recombinant IL-6
(kindly supplied by Dr. Richard Nordan, National Cancer Insti-
tute). The B-9 assay was run as described above except that 2 µg
of antibody against mouse IL-6 was added to the serially diluted
plasma sample and incubated for 1 hour before addition of the B-9
cells.

Stress Paradigm

One method for inducing psychological stress in rats is expo-
sure to an open-field (11,18). The open-field used in these
experiments consisted of a 60" x 38" x 81" high white acrylic spray
finish temperature-controlled chamber (Warren Sherer) illumina-
ted by two fluorescent lights suspended from above. The
temperature within the open-field box (25-26°C) was similar to
that in the rat’s home cages. The experimental protocol for the
stress involved quickly removing the rat from his own small cage
and placing him into the open-field. After the appropriate period of
time, the rat was returned to his cage. Control animals were not
placed in the open-field. In the adaptation study, the rats were
exposed to the open-field for 30 minutes per day for the first seven
days and for 60 minutes for the last three days. To minimize possible
circadian variability, exposure to the open-field occurred only
between 9 a.m. and 3 p.m.

Plasma Samples

In the open-field study, blood was withdrawn from the rats
either prior to or following exposure to the open-field by cardiac
puncture immediately after it was killed by cervical dislocation to
avoid any potential acute stress-induced changes in IL-6 as a result
of anesthesia. Blood was collected into a heparinized syringe in
rats that were removed from their home cages (controls) or from
the open-field stress chamber and surgically dislocated within 20
seconds.

To determine whether anesthesia did in fact have an acute
effect on plasma levels of IL-6, blood was withdrawn from the rats
by cardiac puncture immediately after it was killed by cervical
dislocation following no treatment (control group) or treatment
with either a combination of 70 mg/kg ketamine hydrochloride
(Ketaset, Bristol Laboratories) and 10 mg/kg xylazine (Rompun,
Mobay Corporation) injected intramuscularly, or the gas anes-
thetic methoxyflurane (Pirmax-Moore, Inc.). The blood was
collected as soon as the rats became unconscious, which was about
1-2 minutes.

Plasma was separated by centrifugation of the freshly drawn
blood and stored at −20°C until assayed for IL-6.

Data Analysis

Data are presented as mean ± S.E. Statistical differences were
generally determined by analysis of variance followed either by
Student’s t-tests or paired t-tests corrected in both cases for
multiple comparisons by the method of Bonferroni.

RESULTS

Exposure to an open-field resulted in increases in IL-6 activity
proportional to the period of stress (Fig. 1). Plasma IL-6 activity
in control rats was 40.6 ± 7.2 units/ml. After exposure to the
open-field for 15 minutes, the plasma IL-6 activity was 60.3 ± 5.3
units/ml (p = 0.09). After 30-minutes exposure, plasma IL-6
activity was 105 ± 6.8 units/ml (p = 0.0003) and after 60 minutes
it was 221 ± 17 units/ml (p = 0.0003). We confirmed that the
bioassay was specific for IL-6 by demonstrating that 95% of the
IL-6 activity in rats' plasma following open-field stress was
neutralized by antibody against mouse IL-6.

The effect of repeated exposure to the open-field on plasma
IL-6 activity is shown in Fig. 2. It should be noted that this was not
a longitudinal study of a single group of rats in order to avoid any possible
effect of repeated blood sampling on IL-6. Thus, we only sampled for IL-6
once from each rat.

FIG. 1. Plasma IL-6 activity in control rats (not exposed to open-field), or
rats exposed to open-field for 15, 30 or 60 minutes. Sample size is
indicated in parentheses. p Values were calculated by comparing each of
the open-field-treated groups to the control group using Student’s t-tests
corrected for multiple comparisons with the method of Bonferroni. Each
bar represents a different group of rats. In other words, this was not a
longitudinal study of a single group of rats in order to avoid any possible
effect of repeated blood sampling on IL-6. Thus, we only sampled for IL-6
once from each rat.
Thus we conclude that psychological stress itself (rather than a combination of physical stress) can raise the plasma concentration of IL-6.

Our results also indicate that repeated exposures to open-field results in adaptation in both body temperature and plasma IL-6 secretion. Because we had observed no tendency for adaptation after 7 days of exposure to the open-field, we increased the stress period in order to see whether adaptation of temperature changes or plasma IL-6 can be produced by the combination of multiple exposure and longer period exposure. Therefore, because of the change in protocol on day 8, we do not know whether the adaptation was due mainly to the extra days of exposure (days 8–10) or to the more prolonged exposures (60 minutes) beginning on day 8.

There was a statistically significant correlation between plasma IL-6 and the change in core temperature induced by the stress (Fig. 4). However, despite this correlation, we do not believe that the plasma concentrations achieved are high enough to account for the stress-induced rises in body temperature observed. In a previous study from this laboratory, it has been shown that stress-induced rises in body temperature of rats are not the result of increases in general physical activity (13), and thus we conclude that psychological stress itself (rather than a combination of psychological and physical stress) can raise the plasma concentration of IL-6.

DISCUSSION

Plasma IL-6 activity increased significantly during exposure of rats to an open-field. In another study from this laboratory, it has been shown that stress-induced rises in body temperature of rats are not the result of increases in general physical activity (13), and thus we conclude that psychological stress itself (rather than a combination of psychological and physical stress) can raise the plasma concentration of IL-6.
The study, in which we measured the plasma IL-6 activity and temperature in rats at one and two hours following intraperitoneal injection with $1 \times 10^6$ units of recombinant human IL-6, we found that plasma IL-6 activity rose to $266 \pm 105$ units/ml at one hour and to $193 \pm 32$ units/ml at two hours (12), values similar to those seen in the present stress experiments; these plasma concentrations of IL-6 did not result in a significant rise in body temperature. This analysis, however, applies only to plasma IL-6; it is possible that stress hyperthermia is triggered by local release of IL-6 into the brain (19) or other organ, and that the increase in plasma IL-6 simply reflects leakage of this cytokine into the blood.

The study on the effects of anesthesia on changes in plasma IL-6 activity provided us with the information that the method of collecting blood from rats for assay for IL-6 by injection of the anesthetics ketamine hydrochloride and xylazine, or inhalation of the anesthetic methoxyflurane, caused no greater IL-6 increase than did cervical dislocation.

The finding that psychological stress can increase the plasma concentration of IL-6 is, to our knowledge, the first demonstration that nonphysical stimuli can increase secretion of a cytokine thought to be important in both specific and nonspecific immunity. Other studies (5,6) have shown that exercise is associated with an increase in IL-1, but it is possible that the stimulus in exercise is physical trauma within the exercising muscle. The pathways by which psychological stress increases IL-6 secretion remain to be determined.

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REFERENCES


