Synergism between IL-1β and TNF-α on the activity of the pituitary-adrenal axis and on food intake of rats

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Synergism between IL-1β and TNF-α on the activity of the pituitary-adrenal axis and on food intake of rats. Am. J. Physiol. 268 (Endocrinol. Metab. 31): E551–E557, 1995.—We investigated the effects of separate and combined intraperitoneal administration for 3 days of recombinant human interleukin-1β (IL-1β) and recombinant human tumor necrosis factor-α (TNF) on plasma adrenocorticotropic hormone (ACTH) and corticosterone (B) levels, adrenal weight, food intake, and rectal temperature. Rats were equipped with a jugular cannula for daily blood sampling and with an intraperitoneally implanted Alzet osmotic minipump loaded with either saline, IL-1β (2.0 µg/day), TNF (0.2, 2.0, or 10.0 µg/day), or IL-1β in combination with TNF. Plasma ACTH and B levels and adrenal weight were significantly increased, in a dose-dependent way, by simultaneous infusion of IL-1 and TNF but not by administration of either cytokine alone. Chronic administration of IL-1 alone induced a significant decrease in food intake and a significant elevation of rectal temperature, whereas infusion of only the highest dose of TNF significantly elevated rectal temperature. Coinfusion of IL-1 and TNF induced both effects in a dose-dependent and synergistic way. Our data show that simultaneous infusion of IL-1 and TNF in rats has a synergistic effect on the activity of the pituitary-adrenal axis as well as on food intake and rectal temperature. The existence of two pathways, which act synergistically, may increase the sensitivity of the host to respond to subclinical inflammatory stimuli.

interleukin-1; tumor necrosis factor; hypothalamic-pituitary-adrenal axis; food consumption; body temperature

ACTIVATION OF THE IMMUNE SYSTEM during bacterial infections is accompanied by stimulation of the hypothalamic-pituitary-adrenal axis (HPA axis), and there is increasing evidence that bacterial endotoxin is responsible for the stimulation of both the immune system and the HPA axis during such conditions (1, 20). The activation of the HPA axis by endotoxin is probably mediated by proinflammatory cytokines, especially interleukin-1 (IL-1; see Ref. 21) and tumor necrosis factor (TNF; see Ref. 19). It is thought that these cytokines are also important mediators of other biological effects of endotoxin, including the induction of anorexia, fever, and the acute phase response. Despite these similarities, IL-1 and TNF share no structural homology and recognize different cellular receptors. However, both cytokines are capable of inducing each other’s synthesis (6, 12), and many cells express both IL-1 and TNF receptors, which may contribute to their overlapping biological activities (3).

It has been shown that acute administration of IL-1 (10, 22, 30) or TNF (2, 24, 30) activates the HPA axis in mice and rats, as manifested by increased levels of adrenocorticotropic hormone (ACTH) and corticosterone (B). Del Rey and Bedso (4) demonstrated that IL-1 is more potent than TNF in increasing plasma levels of ACTH and B in rats when administered acutely. There are only a few studies on the effects of chronic administration of IL-1 and TNF on the activity of the HPA axis (8, 16). In the studies from our laboratory, it was shown that continuous infusion of IL-1 (2 and 4 µg/day for 7 days) induced a sustained and dose-dependent stimulation of the HPA axis throughout the infusion period (28), whereas continuous administration of TNF in a dose of 4 µg/day for 7 days did not affect plasma B levels (30).

Many types of cells express receptors for IL-1 and TNF, but both types of receptors have different mechanisms for signal transduction. Therefore the effects of these two cytokines may be additive or synergistic (3). Waage and Espevik (32) demonstrated that IL-1 potentiates the lethal effect of TNF in mice, and synergistic effects of IL-1 and TNF on muscle proteolysis (7) and on plasma ACTH levels (19) were found after coadministration of these cytokines to rats. Okusawa et al. (17) demonstrated in rabbits that the combination of IL-1 and TNF was more potent in inducing hemodynamic and hematological changes typical of septic shock than either agent alone. In contrast, Gelin et al. (9) failed to show synergism between IL-1 and TNF in stimulating the HPA axis. Moreover, Long et al. (13) found that, although both IL-1 and TNF are capable of causing fever when injected into rats, administration of TNF attenuates fever due to injection of IL-1, suggesting antagonism between IL-1 and TNF. Very recently, Mehta et al. (15) showed that low doses of IL-1 and TNF individually stimulate insulin release but in combination cause suppression of insulin release by rat pancreatic β-cells in vitro.

In the light of these controversial data, we investigated in the present study the effects of 3 days continuous infusion of IL-1 and TNF, separately or in combination, on the activity of the HPA axis. Plasma ACTH and B levels were measured daily. In addition, we investigated the effects of the treatments on adrenal weight, daily body weight change, food and fluid intake, and rectal temperature. We show that IL-1 and TNF, when infused simultaneously into rats, synergize to stimulate the HPA axis and to induce dose-dependent changes in body weight, food consumption, and rectal temperature.
METHODS

Materials. Recombinant human (rh) IL-1β was kindly provided by Dr. R. C. Newton (Du Pont Pharmaceuticals, Wilmington, DE). The preparation was supplied in sterile Dulbecco’s phosphate-buffered saline, and endotoxin contamination was negligible (<1 endotoxin unit lipopolysaccharides/mg IL-1). Human TNF-α produced by recombinant DNA technology in Escherichia coli was obtained from Genentech (San Francisco, CA) through the courtesy of Dr. G. Adolf, Ernst-Boehringer-Institut für Arzneimittelforschung (Vienna, Austria). The specific activity of the preparation was *6 × 10^8* U/mg protein on murine L-M cells. The preparation (lot K 9011AX) was supplied in 10 mM sodium phosphate and 200 mM sodium chloride (pH 7). According to the specifications of the suppliers, endotoxin contamination was negligible (<1.2 ng/mg protein, as detected in the limulus amoebocyte lysate assay).

Both IL-1 and TNF were diluted to the desired concentration in sterile pyrogen-free saline just before use. All chemicals used were of analytical grade.

Animals. Male albino Wistar rats (Cpb:WU, 10–12 wk old, 200–220 g) were obtained from the local breeding facility. The animals were individually housed in Plexiglas cages in an artificially lighted room (lights on at 0700; lights off at 1900). Rats were provided commercial rat chow (RMH-TM; Hope Farms, Woerden, The Netherlands) and tap water ad libitum. Room temperature was 23.1 ± 0.2°C.

Experimental procedures. To diminish the stress by the experimental procedures, the animals were handled daily by the experimentator, starting 1 wk before cannulation. Blood was collected from freely moving rats by means of a chronic cannula. Rats were cannulated according to the method described by Steffens (26), with some minor modifications, as described earlier (29). Briefly, under Hypnorm (0.5 ml/kg body wt iv; 10 mg/ml fluanisone and 0.315 mg/ml phentanyl citrate; Janssen Pharmaceutica, Tilburg, The Netherlands)-Dormicium (1.0 ml/kg body wt ip; 5.0 mg/ml midazolam hydrochloride; Hoffmann-La Roche, Mijdrecht, The Netherlands)-atropine (0.025 mg/rat sc; Pharmachemie, Haarlem, The Netherlands) anesthesia, a Silastic cannula (ID 0.5 mm; OD 0.94 mm; Dow Corning, Midland, MI) was inserted into the right external jugular vein and passed down to the atrium. The distal end of the cannula was tunneled subcutaneously and exteriorized through a stab wound on the skin of the head, where it was connected to a hooked stainless steel tube. This assembly was anchored to the skull with three stainless steel screws and acrylic cement. During cannulation, rats were continuously exposed to a gas flow of O_2/N_2/O_2 (each 500 ml/min). To keep the cannula patent, it was filled with a 0.9% NaCl solution containing heparin (500 IU/ml; Organon Teknika, Boxtel, The Netherlands) and polyvinylpyrrolidone (1 g/ml; Merck, Darmstadt, Germany).

Seven or eight days after cannulation, rats were equipped with intraperitoneally implanted osmotic minipumps (1 μl/h for 3 days, model 1003D; Alzet, Palo Alto, CA), which were loaded with either sterile pyrogen-free physiological saline or saline containing IL-1, TNF, or IL-1 in combination with TNF. After loading, the pumps were equilibrated and immersed in saline at 37°C for 3–4 h according to the instructions of the manufacturer. The pumps were implanted intraperitoneally under halothane (ICI Pharmaceuticals, Macclesfield, UK)-O_2/N_2/O_2 anesthesia between 1430 and 1630. The indwelling cannula and the osmotic minipumps were tolerated well by the animals, with no signs of discomfort or infection.

Protocol. A total of eight groups of rats was continuously infused with saline, IL-1, TNF, or with a combination of IL-1 and TNF. One group of rats was infused with 2.0 μg IL-1/day (1 μg IL-1 = 57 pmol IL-1). TNF was infused at a rate of 0.2, 2.0, or 10.0 μg/day. Three other groups were infused with IL-1 (2.0 μg/day) in combination with TNF at a dose of 0.2, 2.0, or 10.0 μg/day (1 μg TNF = 57 pmol TNF). A control group of rats received osmotic pumps filled with 0.9% (wt/vol) NaCl solution. Each group consisted of 6–9 rats.

Food and water intake was estimated daily between 0815 and 0900 by weighing the residual food pellets and water for individual cages. Body weight was measured daily at about the same time (0815–0900), and daily body weight changes were calculated by subtracting the values measured on subsequent days. Body temperature was measured two times a day between 0815 and 0900 and between 1230 and 1430 in conscious hand-held rats by insertion of a thermal probe into the rectum. The probe was connected to a digital temperature monitor (Digital DT100; Elbatron, Kerkruid, The Netherlands). The mean daily rectal temperature for each rat was determined by averaging the morning and afternoon rectal temperatures. At the end of the experiment (day 3) rats were killed by decapitation, and the adrenals were removed, freed of fat, and weighed.

Blood sampling. Blood was collected once a day from freely moving rats for 5 days starting 1 day before implantation of the minipumps (day −1 up to and including day 3). Because of the circadian rhythm in hormone release, blood was withdrawn from the animals every day at about the same time (between 1000 and 1200). Blood samples (1.8 ml) for measurement of plasma levels of ACTH and B were collected on ice in prechilled tubes containing EDTA (45 μl of a 10% (wt/vol) EDTA solution in saline). The samples were gently shaken and spun for 10 min at 1,500 g (4°C). Plasma was separated, and red blood cells were 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 kallikrein inhibitory units aprotinin (Bayer, Leverkusen, Germany) were added.

Hormone measurements. Plasma ACTH and B values were measured by radioimmunoassay, as described by Sweep et al. (28). In the group of rats infused with saline alone and in the group infused with IL-1 in combination with 0.2 μg TNF/day, one rat had to be excluded with respect to hormone measurements because of blood clotting in the jugular cannula.

Statistical analysis. All data are presented as means ± SE of 6–9 rats/group. The effect of infusion of IL-1 in combination with TNF is considered to be synergistic if the effect of combined IL-1 and TNF infusion is significantly larger than the sum of the effects of IL-1 and TNF infusion separately. In the statistical analysis, first the areas under the curves (AUC) were calculated. These were corrected for pretreatment values (day −1 and/or 0) by subtracting the mean of the pretreatment values from treatment values on each day (days 1–3). As it appears that the effect of TNF on the various parameters is directly proportional to the logarithm of the dose, we used the following linear regression model:

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\text{AUC} = a_0 + a_1 \cdot \log \text{TNF} + a_2 \cdot \log \text{IL-1} - \log \text{TNF}
\]

Here *a_0* is an estimate of the effect of chronic administration of saline, *a_1* is an estimate of the effect of chronic infusion of IL-1 alone, *a_2* and *c_0 + c_3* are estimates of the dose-response relationship for the effects of chronic infusion of TNF alone (*a_2*) or TNF in combination with IL-1 (*a_3 + c_3*). So, the coefficient *a_2* of the interaction term is an estimate for the synergism between IL-1 and TNF. Thus testing for a significant dose-response relationship and/or synergism implies testing whether the corresponding *a’s* are equal to zero. In case a significant dose-response relationship was found, Dunnett’s *t*-test was performed to identify which dose of TNF induced a significant change in response compared with the
RESULTS

Effects on plasma ACTH, B, and adrenal weight. Figure 1 shows the effects of 3 days of continuous infusion in rats with saline, IL-1 alone (2.0 μg/day), TNF alone (0.2, 2.0, or 10.0 μg/day), or 2.0 μg IL-1 in combination with 0.2, 2.0, or 10.0 μg TNF/day on plasma levels of ACTH and B. The present experimental conditions (implantation of the minipump, infusion and blood collection procedures), as such, virtually did not alter pituitary-adrenal activity, as demonstrated by stable plasma B levels in animals implanted with saline-filled osmotic pumps.

Preinfusion levels of ACTH and B showed no marked differences between the various groups. ACTH preinfusion values varied between 89 and 137 pg/ml, and preinfusion values of B varied between 24 and 64 nmol/l. Infusion of 2.0 μg IL-1/day caused a small increase in plasma ACTH levels on the 1st day of infusion (+33 ± 14 pg/ml vs. day 0). Administration of 0.2, 2.0, or 10.0 μg TNF/day did not significantly affect plasma ACTH levels. Infusion of IL-1 in combination with increasing doses of TNF (0.2, 2.0, or 10.0 μg/day) induced a dose-dependent increase in plasma ACTH levels compared with ACTH levels in animals infused with IL-1 alone, and a significant dose-response relationship was found with respect to the responses of plasma ACTH (AUC) in the four groups (*P < 0.005). The effect of combined IL-1 and TNF infusion was synergistic (*P < 0.05) compared with the effects of separate IL-1 and TNF infusions. The highest ACTH levels were found on day 1 of infusion (IL-1: 132 ± 21 pg/ml; IL-1 and 0.2 μg TNF/day: 187 ± 12 pg/ml; IL-1 and 2.0 μg TNF/day: 202 ± 29 pg/ml; IL-1 and 10.0 μg TNF/day: 204 ± 21 pg/ml). Plasma ACTH levels declined over days 2 and 3. Due to plasma shortage, plasma ACTH levels of the saline-treated animals could not be measured.

Plasma B levels were not significantly affected in rats continuously infused with either IL-1 alone or TNF alone (AUC, days 1–3) compared with levels in saline-treated rats. Only a small increase in plasma B levels on day 1 of treatment with 2.0 μg IL-1/day or with 10.0 μg TNF/day, compared with saline-treated animals, was observed (IL-1: 130 ± 52 nmol/l; TNF: 104 ± 49 nmol/l;
Simultaneous IL-1 and TNF infusion and the HPA axis

Infusion of IL-1 in combination with 0.2, 2.0, or 10.0 μg TNF/day induced a dose-dependent increase in plasma B levels compared with IL-1 alone; a significant dose-response relationship was found with respect to the responses of plasma B (AUC) in the four groups ($P < 0.0005$). The effect of combined IL-1 and TNF infusion was synergistic ($P < 0.0005$) compared with the effects of separate IL-1 and TNF infusions. The highest B levels were measured on day 1 of infusion (IL-1: $130 \pm 52$ nmol/l; IL-1 and 0.2 μg TNF/day: $262 \pm 52$ nmol/l; IL-1 and 2.0 μg TNF/day: $355 \pm 48$ nmol/l; IL-1 and 10.0 μg TNF/day: $415 \pm 48$ nmol/l). Like the plasma ACTH levels, plasma B levels decreased over days 2 and 3.

Total adrenal weight (summed weight of both adrenal glands) of rats treated for 3 days with 2.0 μg IL-1/day or with increasing doses of TNF alone did not significantly differ from total adrenal weight of rats infused with saline ($40.1 \pm 1.5$ mg). Chronic infusion of rats with IL-1 in combination with 0.2, 2.0, or 10 μg TNF/day induced a dose-dependent increase in total adrenal weight compared with that of rats treated with IL-1 alone. A significant dose-response relationship ($P < 0.0005$) was found with respect to the effects on total adrenal weight in the four groups (IL-1: $39.1 \pm 1.2$ mg; IL-1 and 0.2 μg TNF/day: $44.9 \pm 1.4$ mg, $P < 0.05$ vs. IL-1; IL-1 and 2.0 μg TNF/day: $45.2 \pm 1.1$ mg, $P < 0.05$ vs. IL-1; IL-1 and 10.0 μg TNF/day: $50.8 \pm 1.9$ mg, $P < 0.05$ vs. IL-1). The effect of combined IL-1 and TNF infusion was synergistic ($P < 0.0005$) compared with the effects of separate IL-1 and TNF infusions.

Effects on food and fluid intake and on body weight change. The effects of chronic administration of saline, IL-1 alone, TNF alone, and of IL-1 in combination with different doses of TNF on food and fluid intake and on body weight change are shown in Fig. 2. In saline-infused control rats, there was a slight reduction in both food ($-4.6 \pm 1.3$ g) and fluid ($-4.8 \pm 1.4$ ml) intake 1 day after implantation of the pumps compared with day 0. This decrease is probably due to the anesthesia on the day of implantation of the pumps. Chronic infusion of rats for 3 days with 2.0 μg IL-1/day significantly reduced food intake (AUC, $P < 0.05$), whereas infusion of rats with increasing doses of TNF did not significantly affect food intake during the infusion period compared with saline treatment. Continuous administration of IL-1 in combination with 0.2, 2.0, or 10.0 μg TNF/day induced a pronounced and dose-dependent decrease in food intake compared with food intake by rats infused with IL-1 alone, and a significant dose-response relationship was found with respect to the effects on food intake (AUC) in the four groups ($P < 0.0005$). The effect of combined IL-1 and TNF infusion was synergistic ($P < 0.0005$) compared with the effects of separate IL-1 and TNF infusions. Effects on food intake were maximal on days 1–2 and were only partially restored on day 3.

**Fig. 2.** Effects of 3 days continuous administration of saline, IL-1, or different doses of TNF (left) or IL-1 alone (○), 0.2 μg TNF/day (■), 2.0 μg TNF/day (□), IL-1 and 0.2 μg TNF/day (△), or IL-1 either alone or in combination with increasing doses of TNF (right) IL-1 ( ), IL-1 with 0.2 μg TNF/day (●), IL-1 with 2.0 μg TNF/day (●), IL-1 and 10.0 μg TNF/day (●) on food and fluid intake (top and middle) and on daily body weight change (bottom). Food and fluid intake was estimated by weighing residual food pellets and water for individual cages. Daily body weight change for each rat was calculated by subtraction of daily measured body weights (between 0915 and 0900) on subsequent days. Data are expressed as means ± SE of 5–9 rats. Inset: corrected AUC for days 1–3. Left (separate IL-1 and TNF infusion): saline (a), 2.0 μg IL-1/day (b), 0.2 μg TNF/day (c), 2.0 μg TNF/day (d), or 10.0 μg TNF/day (e). Right (combined IL-1 and TNF infusion): IL-1 (b), IL-1 and 0.2 μg TNF/day (f), IL-1 and 2.0 μg TNF/day (g), or IL-1 and 10.0 μg TNF/day (h). *P < 0.05 vs. saline (left) and IL-1 (right).
Fluid intake was not significantly affected by continuous infusion of rats with 2.0 μg IL-1/day or with increasing doses of TNF (0.2, 2.0, or 10.0 μg/day) compared with fluid intake by saline-infused rats. Infusion of IL-1 in combination with increasing doses of TNF also did not significantly affect fluid intake of the animals compared with that of rats infused with IL-1 alone.

There was a small decrease in body weight on the 1st day of infusion of rats with 2.0 μg IL-1/day or with 2.0 or 10.0 μg TNF/day compared with saline-infused rats. Combined infusion of IL-1 and TNF induced a dose-dependent weight loss of the animals compared with infusion of rats with IL-1 alone, and a significant dose-response relationship was found with respect to the effects on body weight change (AUC) in the four groups (P < 0.0005). The effect of combined IL-1 and TNF infusion was synergistic (P < 0.0005) compared with the effects of separate IL-1 and TNF infusions.

**Effects on rectal temperature.** Figure 3 shows the effects of continuous infusion of saline, IL-1 alone, increasing doses of TNF alone, or of IL-1 in combination with TNF on rectal temperature. Saline-infused rats showed a small decrease in daily rectal temperature compared with preinfusion levels. Chronic administration of IL-1 induced a significant increase (P < 0.0005) in rectal temperature compared with saline infusion. Continuous infusion of increasing doses of TNF (0.2, 2.0, or 10.0 μg/day) induced a dose-dependent amelioration of the decrease in rectal temperature, as seen during saline infusion. A significant dose-response relationship was found with respect to the effects of TNF on rectal temperature (AUC) in the four groups (P < 0.0005). Infusion of rats with IL-1 in combination with TNF induced a dose-dependent increase in rectal temperature compared with the increase in rectal temperature induced by infusion of IL-1 alone, and a significant dose-response relationship was found with respect to the effects on rectal temperature (AUC) in the four groups (P < 0.0005). The effect of combined IL-1 and TNF infusion was synergistic (P < 0.0005) compared with the effects of separate IL-1 and TNF infusions.

**DISCUSSION**

In the present study, it is demonstrated that continuous intraperitoneal infusion of 0.2, 2.0, or 10.0 μg TNF/day had no significant effect on plasma ACTH and B levels and on adrenal weight and that chronic administration of 2.0 μg IL-1/day induced only a slight increase in plasma levels of ACTH and B on the 1st day of infusion. However, continuous infusion of IL-1 in combination with increasing doses of TNF dose dependently induced an increase in plasma levels of ACTH and B and total adrenal weight. Our results demonstrate that IL-1 (2.0 μg/day) and TNF (0.2, 2.0, or 10.0 μg/day) act synergistically to increase plasma levels of ACTH and B and total adrenal weight in rats.

In line with our results, Perlstein et al. (19) recently showed that simultaneous intraperitoneal bolus injections of rhIL-1α (10 ng) and rhTNF-α (1 μg) in mice induced a response of plasma ACTH levels significantly greater than the sum of the ACTH responses to each cytokine injected alone. These workers also demonstrated that the synergistic effect of coinjection of rhIL-1α and rhTNF-α on plasma ACTH levels was completely blocked by pretreatment of the mice with an anti-IL-6 antibody, suggesting that these cytokines synergistically induced IL-6 to produce the ACTH response. Indeed, Shalaby et al. (23) have shown that IL-1 and TNF act synergistically to stimulate IL-6 production in vivo. IL-1 and IL-6 also synergize to stimulate ACTH production; Perlstein et al. (18) showed that rhIL-1α and rhIL-6, when administered in suboptimal doses to mice, synergize to induce an early (30–60 min) response of ACTH, whereas a later (2–3 h) response was similar to that observed after IL-1 administration alone. In contrast, synergism between IL-1 and TNF could not be
demonstrated by Gelin et al. (9), who showed in mice that the effect of intraperitoneal injections of rhIL-1α in combination with rhTNF-α on plasma B levels and on urinary B excretion was as great as the effect of twice daily injections of rhIL-1α alone.

A common problem during infectious diseases is a decrease in appetite. This anorexia is probably a major cause of the body weight loss that occurs with infection. IL-1 (11, 14, 28) and TNF (14, 25, 31) are peptides capable of inducing anorexia when administered in sufficient amounts. There is little known about the effects of combined IL-1 and TNF infusion on food intake and body weight. The present study shows that chronic infusion of rats with IL-1 (2.0 μg/day) significantly decreased food intake, whereas daily body weight change was not significantly affected. Chronic administration of TNF in the doses used in our study (0.2, 2.0, or 10.0 μg/day) had no significant effect on food consumption and on daily body weight change. Continuous infusion of IL-1 in combination with TNF, however, induced a very pronounced and dose-dependent suppression of food intake and of body weight gain during the infusion period. These results are in line with observations by Gelin et al. (9), who demonstrated that twice daily combined injections (40 ng rhIL-1α/day + 45 ng rhTNF-α/day) of mice resulted in a significant decrease in food intake, whereas separate injections of rhIL-1α (40 ng/day) or rhTNF-α (450 ng/day, a dose 10 times higher than in the combined injection) had no effect on food intake.

Another activity of the cytokines IL-1 and TNF is their ability to elevate body temperature. The effects of acute administration of these cytokines on body temperature are well documented (5). We confirm here that chronic infusion of 2.0 μg IL-1/day or 10.0 μg TNF/day for 3 days induces an increase in rectal temperature compared with saline-infused rats, whereas administration of the lower doses of TNF (0.2 or 2.0 μg/day) had no significant effect on rectal temperature. This is in line with previous studies from our laboratory in which it was demonstrated that continuous infusion of 2.0 or 4.0 μg IL-1/day for 1 wk significantly increased rectal temperature (28), whereas chronic infusion of 4.0 or 8.0 μg TNF/day had no effect on rectal temperature (27, 29). In the present study, we show that simultaneous infusion of rats for 3 days with IL-1 combined with increasing doses of TNF induced a dose-dependent increase in rectal temperature, an effect that was clearly synergistic. In this respect, it is of interest that Waage and Espevik (32) found that injection of mice with 0.375 μg recombinant murine TNF-α resulted in hypothermia, whereas injection with 0.5 μg rhIL-1α caused a small increase in body temperature. Combined injection of IL-1 and TNF caused a lowering of body temperature that was of greater magnitude than when TNF was injected alone. Recently, Long et al. (13) reported that the elevation of body temperature in rats induced by an intraperitoneal bolus injection of 10 μg/kg rhIL-1β was significantly decreased by simultaneous injection of 1 μg/kg rhTNF-α, whereas administration of higher doses of TNF (up to 50 μg/kg) slightly, although not significantly, lowered the febrile response to IL-1. TNF itself did not affect body temperature. However, it is difficult to compare our study with the study of Long et al. (13) because we studied rather long-term (1–3 days) effects of continuous infusion of IL-1 and TNF on rectal temperature while Long et al. (13) studied short-term (2–7 h) effects of acute coinjection of IL-1 and TNF on body core temperature.

In summary, we demonstrated that continuous intraperitoneal infusion of rats with 0.2, 2.0, or 10.0 μg TNF/day for 3 days had no significant effect on plasma ACTH and B levels, whereas continuous infusion of 2.0 μg IL-1/day induced only a slight increase in plasma ACTH and B levels on the 1st day of infusion. However, simultaneous infusion of IL-1 with increasing doses of TNF stimulated the HPA axis in a dose-dependent and synergistic way. Infusion of IL-1 alone significantly increased rectal temperature and decreased food intake, and infusion of the highest dose of TNF significantly increased rectal temperature compared with saline infusion. However, simultaneous infusion of IL-1 and TNF induced much greater changes in rectal temperature, food consumption, and body weight change compared with the effects of infusion of each cytokine alone. Although it is not fully established in what ways the apparent redundancy of the IL-1 and TNF systems benefit the organism, the existence of two pathways, which act synergistically, as clearly demonstrated in the present study, may indicate increased sensitivity of the host to respond to subtle inflammatory stimuli (3).

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