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Involvement of protein kinase C and tyrosine kinase in lipopolysaccharide-induced anorexia

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Abstract

Injections of lipopolysaccharide (LPS, 3 μg) into the lateral ventricle elicited anorexia with fever and also decreased body weight in rats. The LPS-induced anorexia was inhibited by intracerebroventicular (icv) injections of anti-interleukin (IL)-1β antibody (Ab), chelerythrine, genistein and tyrphostin 46, but not by icv injections of indomethacin. Consecutive icv injections of orthovanadate and LPS (0.3 μg, a dose of LPS that did not show any effect on food intake, body weight or body temperature) reduced body weight, but did not induce anorexia. On the other hand, icv injections of IL-1β (50 ng) did not influence food intake, although they decreased body weight and produced fever. The IL-1β-induced decrease in body weight was inhibited by icv injections of genistein, but not by icv injections of chelerythrine or indomethacin. These findings suggest that the LPS-induced anorexia is independent of hyperthermia and involves IL-1β generation, tyrosine kinase (TK) and protein kinase C (PKC). This is the first in vivo evidence that activation of TK and PKC induced by LPS is linked to anorexia.

Keywords: Anorexia; Food intake; Body weight loss; Fever; Protein kinase C; Tyrosine kinase; Interleukin-1β; Lipopolysaccharide; Chelerythrine; Genistein; Tyrphostin; Orthovanadate; Indomethacin

1. Introduction

Lipopolysaccharide (LPS), one of the major components of the gram-negative bacteria cell wall, shows multiple effects on food intake, body temperature, blood pressure and so on in vivo (Burgess et al., 1998; Elmquist et al., 1997; Johnson et al., 1997; Langhans, 1996; Plata-Salaman and Borkoski, 1993; Sawada et al., 1995; Scarborough, 1990; Shapira et al., 1994; Tsushima and Mori, 2000). Almost all of them are mediated by increased releases of certain cytokines, neuropeptides or hormones. Prostaglandins (PGs) and IL-1β are well known to be typical mediators of the LPS-induced fever (Elmquist et al., 1997; Tsushima and Mori, 2000; Ushikubi et al., 1998). In LPS-induced anorexia, possible involvement of interleukin (IL)-1β, tumor necrosis factor (TNF)-alpha or cholecystokinin has been shown (Burgess et al., 1998; Daun and McCarthy, 1993; Porter et al., 1998). However, intracellular signal transductions involved in the effects in vivo remain unclear, although in vitro experiments using cultured cells show activation of protein kinase C (PKC), tyrosine kinase (TK) and MAP kinase after LPS administration (Chen et al., 1998; Shapira et al., 1994). Recently, we demonstrated that the activation of TK in the central nervous system (CNS) is a trigger for the LPS-induced release of IL-1β, resulting in hyperthermia (Tsushima and Mori, 2000).

In this study, we investigated the mechanisms underlying the long-term anorexia induced by central injection of LPS. First, the involvements of PGs and IL-1β were examined in anorexia to compare the mediators involved in anorexia and fever. Subsequently, signal transduction in anorexia was examined with protein kinase inhibitors. The LPS-induced anorexia involves IL-1β generation and activation of PKC and TK.

2. Materials and methods

2.1. Experimental protocol

The methods were described in our previous paper (Tsushima and Mori, 2000). Briefly, after male Wistar rats

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(5 weeks old, Japan SLC, Hamamatsu, Japan) were kept at room temperature of 22–24°C and a 12-h light/dark cycle (lights on at 08:00–20:00 hours) for 3 weeks, a guide cannula (AG-8, Eicom, Kyoto, Japan) was stereotaxically fixed in the right lateral ventricle (coordinate: 5.8 mm anterior1 to lambda, 1.8 mm lateral to the midline and 2.8 mm ventral to the skull surface) (König and Klippel, 1963) with dental cement and small screws, the animals were anesthetized with pentobarbiturate (40 mg/kg, ip). During 1 week of recovery, the animals were acclimated to handling and the experimental conditions. With the animal anesthetized with ether, a stainless-steel cannula for drug administration was inserted into the guide cannula at 10:00 hours. The drug cannula was connected to a microsyringe by means of a polyethylene tube containing two kinds of drug solution. The tip of the drug microinjection cannula was located more than 1.0 mm from the tip of the guide cannula. At the same time, a thermistor probe for measurement of rectal temperature was placed in the rectum. After 45 min, a volume of 5% body weight of tap water was orally given to the rats with a rubber cannula, the body weight was measured and the rats were individually moved into an experimental cage. Then, a pretreatment antagonist was introduced into the ventricle. After 45 min, LPS or a cytokine was injected into the ventricle. Rectal temperature and urinary volume were monitored every 15 and 30 min, respectively. After finishing the measurement of rectal temperature and urinary volume (20:00 hours), preweighed commercial chow pellets were given to the rats. Body weight and food intake were measured at 09:00 hours on the following 3 days. The first measured value indicates food intake during the dark phase, 13 h. The results of urinary volume are published elsewhere.

When all of the experiments were finished, the site of the guide cannula was histologically verified under a microscope (Tsushima et al., 1996a,b).

2.2. Drugs

The following drugs were used: LPS (from *Escherchia coli* serotype 0111: B4), tyrphostin 46, indomethacin (Sigma, St. Louis, MO), recombinant rat IL-1β, monoclonal anti-rat IL-1β antibody (Ab) (R&D Systems, Minneapolis, MN), recombinant human TNF-alpha (Pepro Tech EC, London, UK), genistein, sodium orthovanadate (Seikagaku, Tokyo, Japan) and chelerythrine chloride (Research Biochemicals International, Natick, MA). Indomethacin was dissolved in a minimum volume of 0.1 N NaOH and diluted with sterile phosphate-buffered saline (pH 7.4) to the concentration injected. Genistein and tyrphostin 46 dissolved in ethanol were diluted with sterile physiological saline. Sterile phosphate-buffered saline containing 0.1% bovine serum albumin was used as vehicle for the cytokines and the Ab. The other drugs were dissolved in sterile physiological saline.

2.3. Statistical analysis

The results were indicated as the means ± S.E.M. The P values less than .05 were considered as statistical significance with ANOVA followed by post hoc test (Fisher's PLSD test).

3. Results

3.1. LPS-induced effects

LPS injected into the lateral ventricle produced dose-dependent decreases in food intake for the 13-h period after rectal temperature measurement (vehicle: 10.2 ± 1.0 g, $n=9$; 0.3 μg of LPS: 10.3 ± 1.8 g, $n=3$; 3 μg of LPS: 7.1 ± 0.5 g*, $n=7$; 30 μg of LPS: 4.7 ± 1.4 g*, $n=3$, *$P<.05$ vs. the vehicle-injected group). The next 24-h food intake after LPS (3 μg) administration was similar to that of the vehicle-injected group (data not shown). As shown in Fig. 1, the LPS-induced anorexia was inhibited by pretreatment with intracerebroventicular (icv) injections of anti-IL-1β Ab (20 μg). On the other hand, a cyclo-oxygenase inhibitor, indomethacin (15 μg), did not influence the anorexia. However, both anti-IL-1β Ab and indomethacin inhibited the LPS-induced hyperthermia (Table 1). After pretreatment with a PKC inhibitor, chelerythrine (3 μg), or the TK inhibitors, genistein (3.4 μg) and tyrphostin 46 (3 μg), the LPS-induced food intakes were similar to the vehicle-induced intake (Fig. 1). Therefore, the LPS-induced anorexia was completely blocked by the PKC and TK inhibitors.

Icv injections of LPS also produced significant decreases in body weight, compared with that of the vehicle-injected group 24 h after administration (vehicle: −9.5 ± 1.6 g, $n=9$; 0.3 μg of LPS: −8.2 ± 2.6 g, $n=6$; 3 μg of LPS: −21.1 ± 2.0 g*, $n=7$; 30 μg of LPS: −27.3 ± 2.9 g*, $n=3$, *$P<.05$ vs. the vehicle-injected group). Body weight recovered to the predrug level 72 h after LPS (3 μg) administration (−2.20 ± 1.40 g, $n=7$). The LPS-induced decreases in body weight were inhibited by pretreatment with icv injections of anti-IL-1β Ab (20 μg), chelerythrine (3 μg), genistein (3.4 μg) and tyrphostin 46 (3 μg). On the other hand, indomethacin (15 μg) did not influence these decreases (Fig. 2).

The above results showed that the LPS-induced effects possibly involved TK. Activation of TK plays a key role in the LPS-induced fever through the LPS–IL-1β pathway (Tsushima and Mori, 2000). Therefore, the effects on food intake and body weight due to increased tyrosine phosphorylation were investigated after the administration of a tyrosine phosphatase inhibitor. A small amount of LPS (0.3 μg, icv) alone injected into the ventricle did not decrease either body weight (Fig. 2) or food intake (10.3 ± 1.8 g). However, consecutive icv injections of LPS (0.3 μg) after the administration of a tyrosine phosphatase inhibitor, orthovanadate (100 μg, icv), increased body

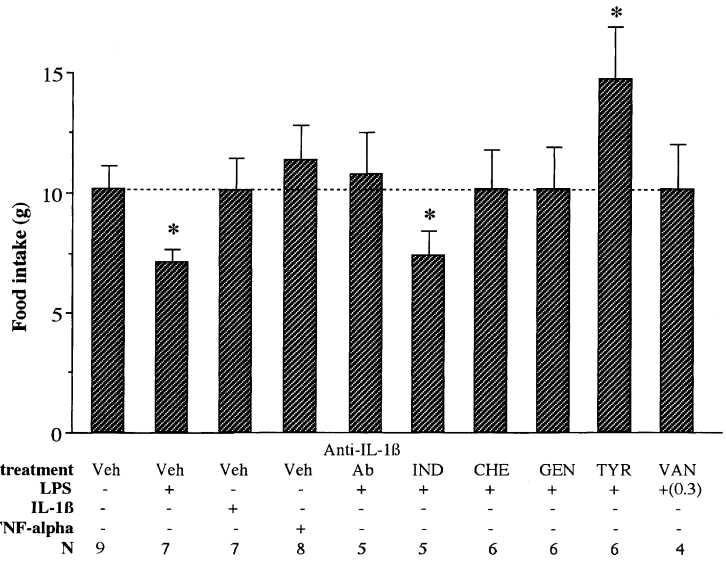

Fig. 1. Food intake induced by icv injection of LPS (3 or 0.3 μg) or cytokines (IL-1β, 50 ng; TNF-alpha, 50 ng) with or without various antagonists. anti-IL-1β Ab: anti-rat IL-1β antibody, 20 μg; IND: indomethacin, 15 μg; CHE: chelerythrine, 3 μg; GEN: genistein, 3.4 μg; TYR: tyrphostin 46, 3 μg; VAN: orthovanadate, 100 μg. Each pretreatment was performed by icv injection 45 min before LPS administration. *N* in the figure shows the experimental number. The columns and bars indicate the means ± S.E.M. of food intake for 13-h period after rectal temperature measurement. * $P < .05$ vs. the vehicle-injected group.

weight loss 24 h after administration and produced slight increases in body temperature (Tsushima and Mori, 2000), although they did not change food intake, compared with that of the vehicle-injected group (Fig. 1).

After icv injections of each of the inhibitors, changes in body weight or food intake were not different from those after injections of vehicle alone (data not shown).

3.2. Cytokine-induced effects

IL-1β (50 ng) injected into the ventricle significantly reduced body weight 24 h after administration, but this effect was relatively feeble, compared with the LPS-induced effect (Fig. 3). Anti-IL-1β Ab and genistein inhibited the IL-1β-induced decrease in body weight, as well as the LPS-induced effect (Fig. 3). Although indomethacin inhibited the IL-1β-induced fever (Table 1), the body weight loss induced by IL-1β was not influenced by the pretreatment with indomethacin. On the other hand, chelerythrine, which inhibited the LPS-induced decrease, did not show any effect on the IL-1β-induced decrease. IL-1β did not influence food intake (Fig. 1).

Icv injections of TNF-alpha did not produce any effects on body weight change or food intake (50 ng, Figs. 1 and 3;

Table 1
Inhibitory effects of anti-IL-1β Ab and indomethacin on the LPS- or IL-1β-induced fever

Drugs	Rectal temperature (°C)
Vehicle + vehicle (9)	0.24 ± 0.21
Indomethacin + vehicle (8)	0.46 ± 0.17
Anti-IL-1β Ab + vehicle (5)	0.17 ± 0.20
Vehicle + LPS (7)	1.06 ± 0.20 *
Anti-IL-1β Ab + LPS (5)	0.32 ± 0.17 **
Indomethacin + LPS (5)	0.35 ± 0.12 **
Vehicle + IL-1β (7)	1.14 ± 0.09 *
Anti-IL-1β Ab + IL-1β (5)	0.41 ± 0.18 **
Indomethacin + IL-1β (5)	0.49 ± 0.15 **

Anti-IL-1β Ab (20 μg) and indomethacin (15 μg) were injected into the lateral ventricle 45 min before icv injection of LPS (3 μg) or IL-1β (50 ng). The values indicate the means ± S.E.M. of changes in rectal temperature 3 h after LPS or IL-1β administration. The experimental numbers are shown in the parentheses.

* $P < .05$ vs. the vehicle-injected group.

** $P < .05$ vs. the LPS- or IL-1β-injected group without the antagonists.

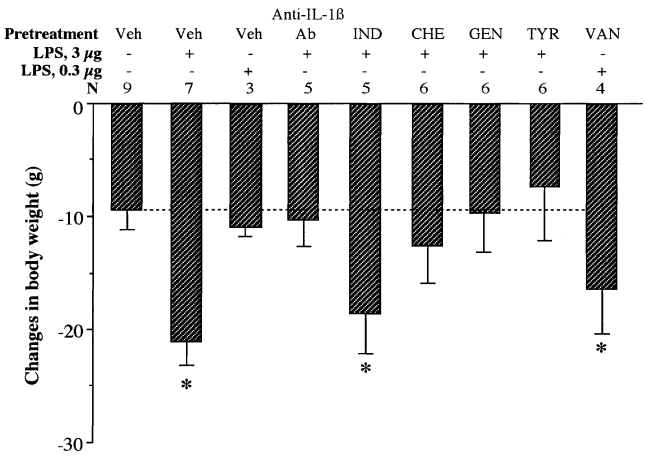

Fig. 2. Changes in body weight induced by icv injection of LPS with or without various antagonists. The doses of the antagonists are shown in the legend of Fig. 1. The columns and bars indicate the means ± S.E.M. of 24-h changes in body weight after LPS administration. *N* in the figure shows the experimental number. * $P < .05$ vs. the vehicle-injected group.

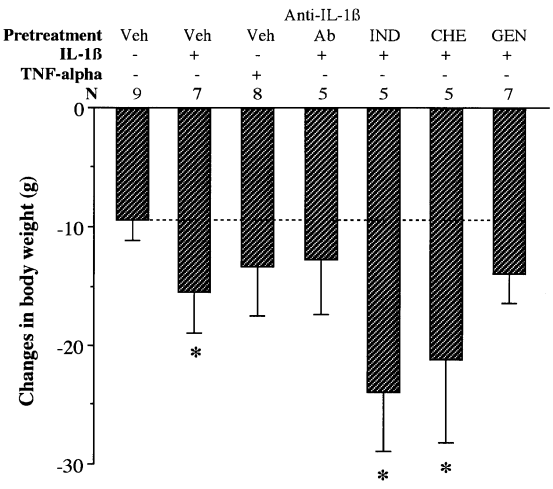

Fig. 3. Changes in body weight induced by icv injection of IL-1β and TNF-alpha with or without various antagonists. The doses of the drugs are shown in the legend of Fig. 1. The columns and bars indicate the means ± S.E.M. of 24-h changes in body weight after administration. *N* in the figure shows the experimental number. * $P < .05$ vs. the vehicle-injected group.

500 ng, data not shown). TNF-alpha (50 ng, icv) also did not change the IL-1β-induced effects on body weight or food intake (data not shown).

4. Discussion

This study demonstrated that the significant long-term anorexia induced by LPS was not at all influenced by indomethacin, although indomethacin did inhibit the LPS-induced fever. This suggests that LPS-induced anorexia is dissociated from thermogeneration in the CNS, as well as in the peripheral nervous system (PNS) (Larson et al., 1996). In addition, the possibility is suggested that anorexia may unrelate to arachidonate metabolites by cyclo-oxygenase. Involvement of PGs cannot be completely excluded from the central mechanisms of anorexia. It is unknown whether indomethacin blocks cyclo-oxygenase for hours, although it inhibited the LPS-induced fever at least for 8 h (Tsushima and Mori, 2000). Anorexia induced by peripheral injections of LPS has been demonstrated to be inhibited by indomethacin (Langhans et al., 1989; Swiergiel et al., 1997). Therefore, in the PNS, LPS-induced anorexia involves cyclo-oxygenase products from arachidonic acid. Frequently, LPS produces the same phenomena in vivo after both central and peripheral injections. It is not unusual that the mechanism underlying the same effect by the two administration routes are different from each other. Indomethacin did not inhibit the decrease in body weight induced by LPS. The decrease in body weight after vehicle administration is assumed to be due to the experimental time schedule in that the animals were removed from food during measurement of body temperature and urinary volume. The body weight of rats given food immediately after vehicle administration without these measurements did not change significantly (-2.0 ± 1.5 g, $n = 5$; unpublished data).

In this study, the LPS-induced anorexia was inhibited by anti-IL-1β Ab. IL-1β-converting enzyme-deficient mice are shown to resist long-term anorexia induced by LPS (Burgess et al., 1998). LPS increases the IL-1β mRNA level and releases IL-1β in the CNS (Elmquist et al., 1997; Gayle et al., 1999; Ilyin et al., 1998; Quan et al., 1994). Taken together, IL-1β is probably necessary for the LPS-induced anorexia. On the other hand, IL-1β alone did not show any effect on long-term food intake (this study and Burgess et al., 1998), although IL-1β produces short-term anorexia (discussed below). Therefore, there remains the possibility that the LPS-induced anorexia may involve any mediator(s) combined with IL-1β. TNF-alpha, IL-6, IL-8 and leptin may be raised as candidates of the mediators. These are released by LPS administration and produce anorexia (Calapai et al., 1998; Finck and Johnson, 1999; Finck et al., 1997, 1998; Sonti et al., 1996). In this study, TNF-alpha did not decrease food intake in the dark phase by itself or by coinjection with IL-1β. Porter et al. (1998) demonstrate that TNF-alpha tolerance blocks anorexia induced by intraperitoneal injections of LPS. In the PNS, it is suggested that TNF-alpha plays a major role in anorexia. On the other hand, it is reported that leptin increases the release of IL-1β, resulting in anorexia (Luheshi et al., 1999). Decreased release/generation of neuropeptide Y (NPY), the strongest potentiator of feeding (Inui, 1999a), is not involved in the LPS-induced anorexia. LPS does not decrease the NPY mRNA content in the hypothalamus (Inui, 1999a) or the NPY concentration in the plasma (Wang et al., 1992).

Several laboratories have demonstrated that IL-1β and TNF-alpha produce anorexia (Burgess et al., 1998; Plata-Salaman et al., 1988; Porter et al., 1998; Sellami and de Beaurepaire, 1995), which is inconsistent with our findings. The reasons for this discrepancy remain unclear, but may be related to the following: (1) Differences in experimental time schedule: In the studies referred to, the cytokines are administrated to fed animals just before the dark phase, and food intake for relatively short periods of time, such as 2 or 4 h, is measured. Our rats were given food pellets at 20:00 hours (light-off time) after measurement of body temperature and urinary volume under a nonfed condition for 9 h after drug administration, and then the long-term food intake for 13 h from 20:00 hours, in the dark phase, was observed. These differences in time schedule may cause different effects of drugs in vivo. The effects of the cytokines on food intake probably are of short duration. Our hypothesis is that the long-term anorexia induced by LPS is mediated through unknown factor(s) that is (are) released by LPS itself and/or by increased generation of cytokines after LPS administration, although a large part of the short-term anorexia seems to be mediated through these cytokines. (2) Differences in the drug administration route: Central/peripheral injections or

the lateral/third ventricle injections also may cause the discrepancy in the results. Many brain regions influencing food intake are adjacent to the lateral and third ventricles. It is demonstrated that IL-1β increases food intake in the paraventricular nucleus of the hypothalamus, but decreases it in the perifornical area and the anterior floor of the hypothalamus (Sonti et al., 1996). Mediators released after peripheral injections of LPS, IL-1β or TNF-alpha will be different in quality, quantity, time and space from those after central injections. These have not been examined sufficiently. (3) Differences in sensitivity to IL-1β: Sensitivity to LPS may differ greatly, depending on species and strains of the animals investigated. Similarly, sensitivity to cytokines may be different. Two or five nanograms of IL-1β (icv), which is one-twenty-fifth or one-tenth of our dose decreased food intake (Burgess et al., 1998; Sellami and de Beaurepaire, 1995).

The inhibitory effects of chelerythrine, genistein and tyrphostin 46 on LPS-induced anorexia suggest the involvement of both PKC and TK. Our previous paper demonstrates that the activation of TK plays an essential role in IL-1β generation in LPS-induced hyperthermia and the activation of PKC is not involved in the LPS–IL-1β pathway resulting in hyperthermia (Tsushima and Mori, 2000). As described above, LPS-induced anorexia will involve at least two mediators, IL-1β and unknown mediator(s). The former needs the activation of TK, but not PKC. Therefore, the activation of PKC will be necessary for the other mediator(s). This is supported by the result that coinjections of orthovanadate and LPS decreased body weight without any effect on food intake, which is the same as the IL-1β-induced effect. Feeding behavior is influenced by many kinds of neurotransmitters/modulators (orexin, galanin, NPY, glucagon-like peptide, bombesin, leptin, etc.) (Inui, 1999b) and various factors (taste, smell, locomotor activity, etc.) (Langhans, 1996). Moreover, it is controlled by many neurons in some brain regions (the dorsomedial hypothalamus, the ventromedial hypothalamus, the hypothalamic paraventricular nucleus, etc.) (Inui, 1999a). Therefore, further studies are needed to determine the steps and brain region(s) activating PKC and TK after LPS administration resulting in anorexia. Nevertheless, the activations of PKC and TK are involved in the mechanism of LPS-induced anorexia.

LPS with pretreament with tyrphostin 46 increased the food intake over baseline, but this phenomenon did not appear after LPS administration with pretreatment with the other antagonists. This will induce a small decrease in body weight loss. Tyrphostin 46 alone showed a tendency to increase food intake, but this was not significant (12.8 ± 1.5 g, $n = 5$). Because the other TK inhibitor, genistein, simply brought the LPS-induced anorexia back to the control level, increased food intake after tyrphostin 46 and LPS may correlate with any protein kinase except TK or different types of TK that genistein blocks.

In conclusion, LPS-induced anorexia in the CNS is independent of hyperthermia and involves IL-1β generation, TK and PKC. This is the first in vivo evidence that the activation of PKC and TK induced by LPS is linked to the anorexia.

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