Korean J Physiol Pharmacol
Vol 5: 133-138, April, 2001

Effects of Pretreatment of Serotonin Synthesis Inhibitor p-chlorophenylalanine on Lipopolysaccharide-induced Anorexia in Rats

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In the present study, we investigated the effect of pretreatment of p-chlorophenylalanine (PCPA), inhibitor of serotonin synthesis, on lipopolysaccharide (LPS)-induced anorexia in rats. First of all, effects of PCPA injection on food intake and body weight in rats were investigated. During 4 days of PCPA injection (300 mg/kg BW once a day), food intake was decreased by 33% and daily gain in body weight was inhibited compared with controls. Twenty-four hours after last PCPA injection, food intake and gain in body weight returned toward almost normal. Pair-feeding to PCPA (PCPAP) injection revealed that body weight of rats in PCPA group was not different from rats in PCPAP groups. To quantify the effects of LPS on food intake and body weight, we administered varying doses (10, 100, 500 μg/kg BW) of LPS to rats. LPS induced a reduction of food intake and weight loss in a dose dependent manner compared with controls. To evaluate the effects of PCPA pretreatment on LPS injection, rats were treated with PCPA for 4 days (300 mg/kg BW once a day), which was followed by LPS injection for 2 days (500 μg/kg BW once a day) (PCPA+LPS group), while rats in LPS group had injections with normal saline instead of PCPA for 4 days, which was followed by LPS administration. Rats in control group received 0.9% NaCl for 6 days. LPS decreased food intake by 80% and inhibited gain in body weight, while PCPA pretreated rats showed normalized food intake and gain in weight during the days of LPS injections compared with controls. In conclusion, pretreatment of PCPA prevented LPS-induced anorexia.

Key Words: Serotonin, Anorexia, Lipopolysaccharide, Rats

INTRODUCTION

Anorexia, a syndrome of depressed appetite, and weight loss complicate many diseases, including chronic infection (Kent et al, 1992b; Cooney et al, 1997), cancer (Tisdale, 1993), and AIDS (Grunfeld & Feingold, 1992). Although both decreased caloric intake and an increased metabolic rate have been observed in patients with chronic illness, recent data indicate that decreased caloric intake plays a major role in infection-induced weight loss. For example, in AIDS, which is characterized by hypermetabolism and disturbances in lipid metabolism (Grunfeld & Feingold, 1992), weight loss only occurs in the presence of decreased caloric intake (Grunfeld et al, 1992; Macallan et al, 1995). However little is known about the mechanism of anorexia.

Lipopolysacchride (LPS), endotoxin from gram-negative bacteria cell walls, is well known to produce similar responses to infection, when injected intraperitoneally (Movat et al, 1987). Many physiological effects of LPS are mediated by cytokines (McCarthy et al, 1984; Kent et al, 1992a), which are shown to induce anorexia and cachexia (Gayle et al, 1998). Possible mechanisms involved in these responses are both direct effects on the feeding-regulating center in the brain (Fantino & Wieteska, 1993; Plata-Salaman & Borkoski, 1994) and/or act on peripheral tissues to induce hormones, which are known to regulate appetite (McCarthy et al, 1986; Bodnar et al, 1989).

Recent data indicate that administration of LPS produces increased metabolism of hypothalamic serotonin (Dunn, 1992; MohanKumar et al, 1999), a

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neurotransmitter, which powerfully inhibits feeding in rodents when injected either into the various hypothalamic regions or intraperitoneally (Leibowitz et al, 1988). Depletion of serotonin in the brain inhibits LPS-induced increase of hypothalamic corticotrophin releasing factor (CRF) (Laflamme et al, 1999). CRF is also known to decrease food intake (Hotta et al, 1991). Furthermore, serotonin antagonists inhibit an increase of plasma corticosterone by LPS administration (Guo et al, 1996). Therefore it can be inferred from these data that hypothalamic serotonin might play a major role in LPS-induced anorexia.

Para-chlorophenylalanine (PCPA) has an inhibitory effect on tryptophan hydroxylase, the enzyme involved in serotonin synthesis (Koe & Weissman, 1966). PCPA injections for 3 days produces 90% depletion of serotonin in brain (Harvey et al, 1975). As one of the causes of anorexia by LPS might be ascribed to increased serotonin metabolism, administration of PCPA is expected to attenuate the anorexia through serotonin depletion.

Therefore, to investigate the effects of pretreatment of PCPA on LPS induced anorexia, we administered LPS intraperitoneally into PCPA-pretreated rats and measured food intake and body weight.

METHODS

Animal and drugs

Male Sprague-Dawley rats weighing 180 g were purchased from Jung-Ang Lab Animal (Korea) and housed in an animal unit of College of Medicine, Yeungnam University for 4 days before starting the experiments. Standard chow diet was fed and water was accessed *ad libitum*. DL-*p*-chlrolophenylalanine methyl ester hydrochloride (PCPA) (Sigma, USA) and LPS 055 : B5 (Sigma, USA) were dissolved in 0.9% NaCl and injected intraperitoneally into rats. After experiments, rats were sacrificed by carbon dioxide inhalation.

Experiment of PCPA effect on food intake and body weight

Rats were randomly allocated to one of three groups (n=7) and injected with either 0.9% NaCl (control and PCPA pair-feeding groups) or PCPA (300 mg/kg BW) (PCPA group) once a day for 4 consecutive days. A measured amount of food was placed in the rodent food container and the amount consumed was measured 24 h after injection in control and PCPA groups. Rats in PCPA pair-feeding group (PCPAP) were pair fed to rats in PCPA group. Body weights were measured everyday.

Experiment of LPS effect on food intake and body weight

Sixteen rats were randomly allocated to one of four groups (n=4) and injected with 0.9% NaCl, 10 μg/kg BW, 100 μg/kg BW, or 500 μg/kg BW. All rats accessed food *ad libitum*. Food intake and body weight were measured everyday.

Experiment of effect of PCPA pretreatment on LPS-induced anorexia and body weight

Twenty one rats were randomly allocated to control, LPS or PCPA+LPS groups (n=7). Rats in PCPA +LPS group were treated with PCPA for 4 days (300 mg/kg BW once a day), which were followed by LPS injection for 2 days (500 μg/kg BW once a day), while rats in LPS group were injected with normal saline for 4 days instead of PCPA, which was followed by LPS administrations. Rats in control group received 0.9% NaCl for 6 days. All rats accessed food *ad libitum*. Food intake and body weight were measured everyday.

Statistical analysis

The results were expressed as mean±SEM. Differences between the groups were analyzed with a one-way analysis of variance (ANOVA) followed by LSD t-test using SPSS system.

RESULTS

PCPA did not increase food intake and body weight

During the days of injections, PCPA reduced food intake by 33% and inhibited gain in body weight compared with controls. Twenty-four hours after last PCPA injection, food intake returned almost to normal (Fig. 1). We calculated cumulated food intake from 24 h after last injection for 16 days and it was not different between the two groups (Fig. 2). Gain

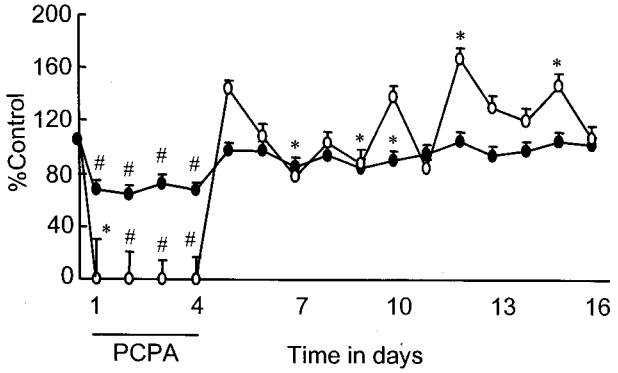

Fig. 1. Percents control of food intake (closed circle) and gain in body weight (open circle) in p-chlorophenylalanine (PCPA) treated rats. *$p<0.05$ and #$p<0.001$, vs controls.

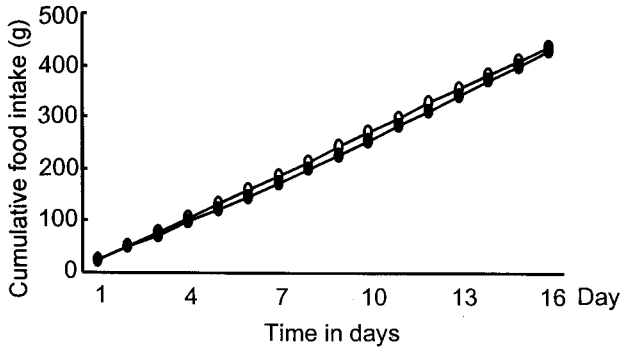

Fig. 2. Cumulative food intake after last injection of p-chlorophenylalanine (PCPA) for 16 days in control (open circle) and PCPA-treated (closed circle) rats.

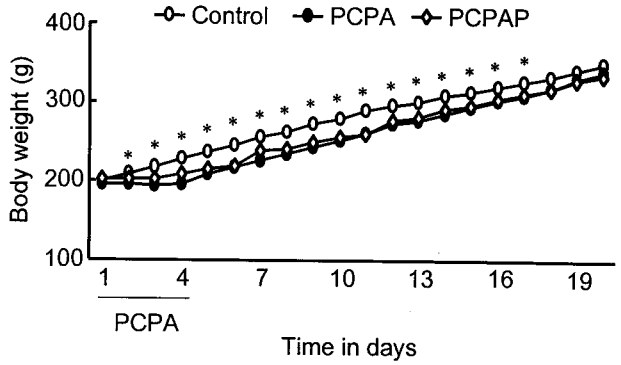

Fig. 3. Changes of body weight in control, p-chlorophenylalanine (PCPA) treated, and PCPA pair-feeding (PCPAP) rats. *$p<0.05$, vs PCPA and PCPAP.

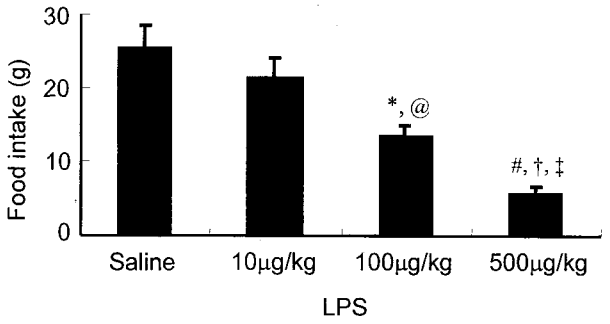

Fig. 4. Effect of various concentrations of a lipopolysaccharide (LPS) injection on food intake for 24 h in rats. *$p<0.05$ and #$p<0.001$, vs controls, @$p<0.05$, vs. 10 μg/kg LPS, and †$p<0.01$ and ‡$p<0.05$, vs 10 μg/kg LPS and 100 μg/kg LPS, respectively.

in body weight also returned to normal 24 h after last PCPA injection (Fig. 1). Pair-feeding to PCPA injection revealed that body weight of rats in PCPA group was not different from rats in PCPAP groups (Fig. 3).

LPS produced anorexia and weight loss

To quantify the effect of LPS on food intake and body weight we administered varying doses of LPS to rats. The lowest dose of LPS tested (10 μg/kg) induced a 16% reduction in food intake but there was no statistical difference compared with controls. Dosages of 100 and 500 μg/kg BW LPS significantly decreased food intake compared with control (50% and 21% of control, respectively) and 10 μg/kg LPS (62% and 25% of 10 μg/kg BW LPS, respectively). Overall LPS reduced food intake in a dose-dependent manner (Fig. 4). Though gain in body weight in rats treated with 10 μg/kg LPS decreased compared with controls, this did not reach significance. Rats given higher dosage of LPS lost weight significantly compared to control and 10 μg/kg LPS groups. Loss of body weight had a similar tendency with food intake (Fig. 5).

PCPA pretreatment prevented LPS-induced anorexia and weight loss

Without PCPA pretreatment, LPS decreased food intake by 80% and 45% with the first and the second injections compared with controls, respectively. Food intake in rats in PCPA+LPS group was reduced by PCPA injection and tended to returned toward normal in spite of following LPS injections (Fig. 6). General tendency in changes of the gain in body weight was similar to that of food intake in both groups. However, while the first LPS injection induced severe loss

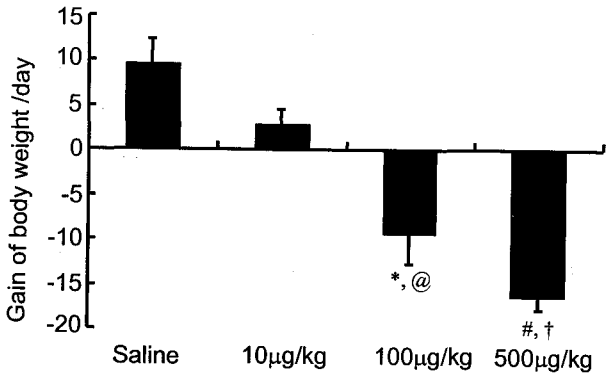

Fig. 5. Effect of various concentrations of a lipopolysaccharide (LPS) injection on gain in body weight for 24 h in rats. *$p<0.05$ and $^{\#}p<0.001$, vs controls, $^{@}p<0.05$, vs 10 μg/kg LPS, and $^{\dagger}p<0.01$ vs 10 μg/kg LPS.

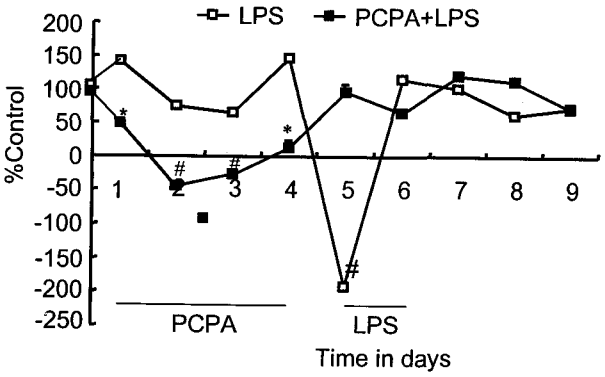

Fig. 7. Percents control of gain in body weight in rat injected with lipopolysaccharide (LPS) and in rats treated with p-chlorophenylalanine (PCPA) and LPS. *$p<0.05$ and $^{\#}p<0.001$ vs controls.

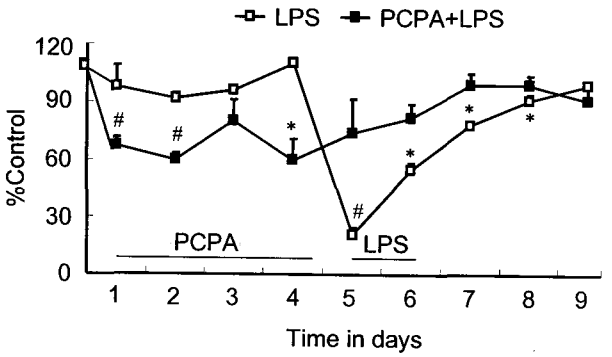

Fig. 6. Percents control of daily food intake in rat injected with lipopolysaccharide (LPS) and in rats treated with p-chlorophenylalanine (PCPA) and LPS. *$p<0.05$ and $^{\#}p<0.001$, vs controls.

of body weight (200%) in LPS group, gain in body weight completely normalized following the second injection (Fig. 7).

DISCUSSION

PCPA, the enzyme involved in serotonin synthesis and has an inhibitory effect on tryptophan hydroxylase (Stokes et al, 2000), has been used to lower serotonin level (Reader & Gauthier, 1984). It was anticipated that PCPA administration would lower serotonin level in the CNS and correspondingly would increase food intake. However the results of the present study were considerably different from the anticipated one.

Food intake was transiently reduced during the days of PCPA injection, and then returned toward normal but was not increased more than that of control. There are controversies in previous studies about the effects of PCPA on food intake and body weight. While PCPA (100 mg/kg BW) injected intraperitoneally at 2-day intervals for 3 days does not increase food intake in rats (Gietzen et al, 1987), centrally injected PCPA has consistently produced an increase of food intake and body weight (Breish et al, 1976; Fuller et al, 1987). The mechanisms involved in this difference are not known but influence of PCPA on peripheral tissues might be the candidate. According to previous studies, 3-day treatment with PCPA strongly suppresses the spontaneous and motilin-induced contraction in the stomach of the dog (Haga et al, 1996). PCPA also influences the pancreas to impair exocrine secretion (Metz & Forssmann, 1976; Chen et al, 1996). It is possible that an increase of food intake by serotonin depletion in the hypothalamus could be offset by a decrease of food intake, which might be caused by the above reason.

Contrary to PCPA, effects of LPS on food intake and body weight were consistent with previous studies (Grunfeld et al, 1996). LPS administration decreased food intake and body weight in a dose dependent manner. However PCPA-pretreated rats showed normal food intake and gain in body weight in spite of LPS administration. Consistent with our result, Ballinger et al (2000) reported that rats with experimental colitis show a decrease in food intake and an increase in 5-HT concentration in hypothalamus, which are partially reversed by pretreatment of PCPA. Although not measured in the present study,

a lower dose than 300 mg/kg BW PCPA for 3 days produces 90% depletion of forebrain serotonin for 2 weeks (Koe & Weissman, 1966; Harvey et al, 1975). Therefore, we suggest that preventive effect of pretreatment of PCPA on LPS-induced anorexia was mediated by serotonin depletion in the brain. These results also implicate that LPS-induced anorexia in rats might be caused by increased serotonin metabolism but further studies are needed to clear it.

In conclusion, pretreatment of PCPA could prevent LPS-induced anorexia.

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