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Preventive Effect of Serotonergic Drugs on LPS-Induced Acute Anorexia in Rats

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The aim of the present study was to determine whether serotonergic drugs could reverse lipopolysaccharide (LPS)-induced anorexia in rats. LPS (500 μg/kg body weight) and all serotonergic drugs, except for 8-OH-DPAT (subcutaneous), were injected intraperitoneally into Sprague-Dawley rats. Without the LPS injection, 8-OH-DPAT (1A agonist), metergoline (1/2 antagonist), and mianserin (2A/2C antagonist) exerted no effects on food intake at any of the doses tested, but ketanserin (2A antagonist) caused an increase of food intake at 4 mg/kg. RS-102221 (2C antagonist) reduced food intake at 2 and 4 mg/kg. LPS reduced food intake 1 hour after injection, and food intake remained low until the end of measurement period (24 hours) ($p < 0.05$). Pretreatment of rats with 8-OH-DPAT partially recovered of cumulative food intake at all measured times (2, 4, 6, 8, and 24 hours after LPS injection). Pretreatment with metergoline resulted in a partial recovery of cumulative food intake at 2, 4, 6, and 8 hours, but not at 24 hours. Ketanserin caused partial recovery at 2 and 4 hours only. Mianserin and RS-102221 had no effects on LPS-reduced food intake. A variety of serotonergic drugs ameliorated anorexic symptoms, which suggesting that the serotonin system plays a role in LPS-induced anorexia.

Key Words: Lipopolysaccharide, Anorexia, Serotonin, Serotonergic drug, Rat

INTRODUCTION

Anorexia and body weight loss are common complications in many diseases, including infections (Kent et al, 1992; Cooney et al, 1997) and inflammation (Simons et al, 1999). Despite many metabolic changes which occur during infection and inflammation, reduced caloric intake plays a major role in weight loss. For example, despite hypermetabolism associated with AIDS patients (Grunfeld & Feingold, 1992), weight loss occurs only in the presence of decreased caloric intake (Grunfeld et al, 1992; Macallan et al, 1995).

Lipopolysaccharide (LPS), when injected intraperitoneally, is known to generate responses reminiscent of infection (Movat et al, 1987). Many of the physiological effects of LPS are mediated by cytokines, and ample evidence indicates that a variety of cytokines play a central role in anorexia due to infection and LPS (McCarthy et al, 1984; Kent et al, 1992a). One of the possible mechanisms underlying anorexia associated with infection or LPS is direct modulation of the feeding-regulating center of the brain (Fantino & Wieteska, 1993; Plata-Salaman & Borkoski, 1994). This hypothesis is supported by the fact that peripherally injected LPS or cytokines tend to modulate energy-regulating hypothalamic peptides, including neuropeptide Y (NPY), galanin, pro-opiomelanocortin (POMC) (Sergeyev et al, 2001) and serotonin (Dunn, 1992; Lavicky & Dunn, 1995).

Serotonin exerts a profound inhibitory effect on feeding in rodents, when injected either into various hypothalamic regions or intraperitoneally (Leibowitz et al, 1988; Sugimoto et al, 2002; Choi et al, 2003). In recent years, a number of 5-HT receptors have been identified (Hoyer et al, 1994), and some of these have been implicated in the modulation of food intake (Sharp & Mjorth, 1990; Grignaschi et al, 1996; Bonhaus et al, 1997). 5-hydroxyindoleacetic acid, a serotonin metabolite in the brain, tends to be elevated as a result of administration of either LPS or cytokines. This indicates that increased serotonin metabolism results in LPS-induced anorexia.

We hypothesized, therefore, that the administration of LPS might affect changes in serotonin levels, thereby inducing anorexia, and that a countermodulation of these serotonin levels might prevent LPS-induced anorexia. Therefore, the objective of the present study was to determine whether the peripheral administration of various serotonergic drugs would reverse LPS-induced anorexia in rats.

METHODS

Male Sprague-Dawley rats (200 g body weight) were pur-

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ABBREVIATIONS: LPS, lipopolysaccharide.

chased from Jung-Ang Lab Animals (Seoul, Korea), and housed in the animal unit of the College of Medicine at Yeungnam University. Two weeks before commencing the experiment, rats were housed in a group cage in a room which was on a 12:12-h light/dark cycle (lights-off at 11:00 and on at 23:00). Four days before the experiment, the rats were transferred to separate cages, and were fed on a standard chow diet, and given *ad libitum* access to water. Food intake was monitored by manual weighing and spillage was taken into account. This study was conducted in accordance with the guidelines for the care and use of laboratory animals provided by Yeungnam University, and all experimental protocols were approved by the ethical committee of Yeungnam University.

LPS 055:B5 was purchased from Sigma (St. Louis, MO, USA), and all serotonergic drugs used in this experiment were purchased from Tocris Cookson (Bristol, UK), Ltd.

Mianserin, metergoline phenylmethyl ester, RS 102221, and ketanserin were intraperitoneally injected into rats, and 8-OH-DPAT was injected subcutaneously. The dose of LPS was determined by measuring the change of food intake after intraperitoneal injection of various doses of LPS. LPS reduced food intake in a dose dependent manner, and average food intake was 80%, 56% and 25% of food intake before 10 μg/kg, 100 μg/kg, and 500 μg/kg LPS injection, respectively. At the dose of 10 μg/kg, LPS was not significantly enough to reduce food intake in all rats (some rats showed 90% of food intake before LPS injection), and 100 μg/kg of LPS reduced food intake by varying extent, (30~80% of food intake before LPS injection). At the dose of 500 μg/kg, LPS reduced food intake with narrow range of variation (15~35% of food intake before LPS injection), therefore, we used this dose for the experiments (Fig. 1).

The effects of serotonergic drugs on normal food intake in rats were measured as the first step of experiment. Food was removed at 10:00, and the drugs were injected at 10:30, 30 minutes before the light off. The food was returned into cages at 10:50. 8-OH-DPAT was injected subcutaneously at doses of 0.1, 0.2, 0.4, and 1 mg/kg. The other drugs were intraperitoneally injected, at doses of 1 and 5 mg/kg for mianserin and metergoline, and at doses of 1, 2, and 4 mg/kg for ketanserin and RS-102221. In order to determine the effects of these serotonergic drugs on LPS-induced anorexia, doses of 1, 5, 5, 1 and 4 mg/kg were used for 8-OH-DPAT, mianserin, metergoline, RS-102221, and ketanserin treatments, respectively. These doses were determined to be the maximal dose which did not reduce normal food intake. The food was removed at 10:00 and serotonergic drugs were injected at 10:20. LPS was then injected intraperitoneally at a dose of 500 μg/kg, 30 minutes after the administration of serotonergic drugs (Lenczowski et al, 1997; Sugimoto et al, 2002). The food was returned into cages right away after LPS injection. The control rats were injected with normal saline rather than LPS. Food intake was measured 2, 4, 6, 8, and 24 hours after the administration of the drugs.

Statistical analysis

All results were expressed as means±SEM. The differences between the two groups were analyzed via Student's t-tests. The SPSS system was used for statistical analysis.

RESULTS

LPS reduced food intake beginning 1 hour after injection, and cumulative food intake was also found to decrease until 24 hours after injection (Fig. 2). Plasma TNF-alpha levels were elevated at 0.5 and 2 hours after LPS administration

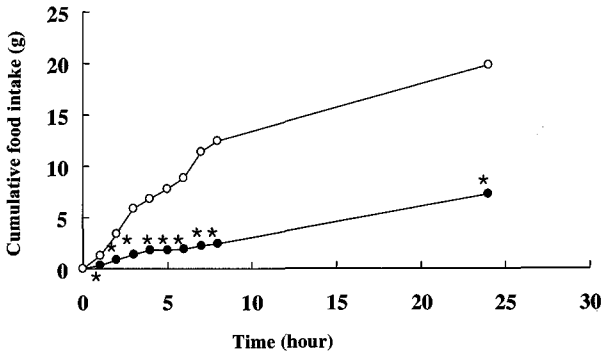

Fig. 2. Cumulative food intake after lipopolysaccharide (LPS) injection (500 μg/ kg, i.p). Control rats were injected with normal saline. Food intake was significantly reduced in LPS-injected rats (closed circle), compared to control rats (open circle), between 1 to 24 hours after injection. *p<0.05 vs control.

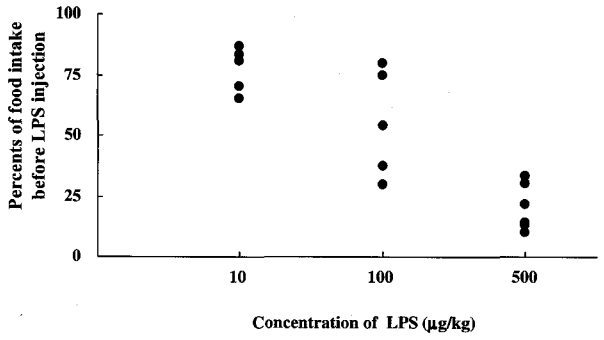

Fig. 1. Effects of various doses of lipopolysaccharide (LPS) on food intake. Food intake was represented as percent of food intake before LPS injection.

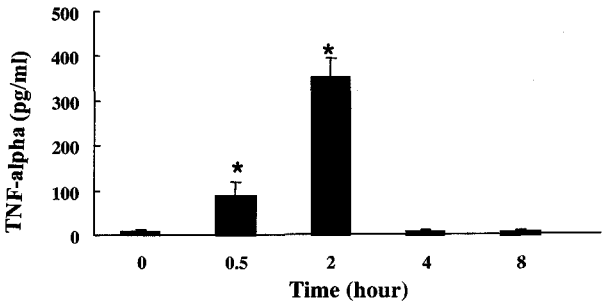

Fig. 3. Time-dependent changes in plasma concentration of tumor necrosis factor-alpha (TNF-alpha) after lipopolysaccharide (LPS) injection (500 μg/ kg, i.p). *p<0.05 vs 0 hour.

and reached maximal levels at 2 hours (Fig. 3). We initially determined the effects of the serotonergic drugs on normal food intake. Food intake remained unchanged after the administration of all doses of 8-OH-DPAT (Fig. 4), metergoline (Fig. 5), mianserin (Fig. 6), and ketanserin tested (Fig. 7). However, RS-102221 (Fig. 8) administration resulted in a decline of food intake at dosages of 2 and 4 mg/kg. This effect proceeded in a dose-dependent manner. Secondly, we determined the effects of pretreatment with these drugs on LPS-induced anorexia. Cumulative food intake, which decreased as the result of LPS treatment, partially recovered as the result of 8-OH-DPAT pretreatment, at 2, 4, 6, 8, and

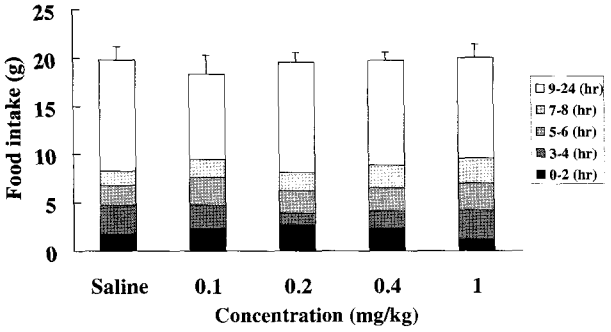

Fig. 4. Effects of subcutaneous injection of 8-OH-DPAT on food intake. Rats were injected with either saline or one of various doses (0.1, 0.2, 0.4, and 1 mg/kg) of 8-OH-DPAT. There were no statistical differences between the groups during each time interval and 24 hours.

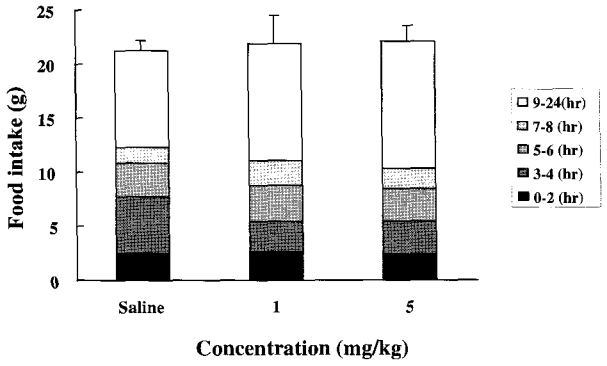

Fig. 5. Effects of intraperitoneal injection of metergoline on food intake. Saline was injected into control rats. There were no statistical differences between the groups during each time interval and 24 hours.

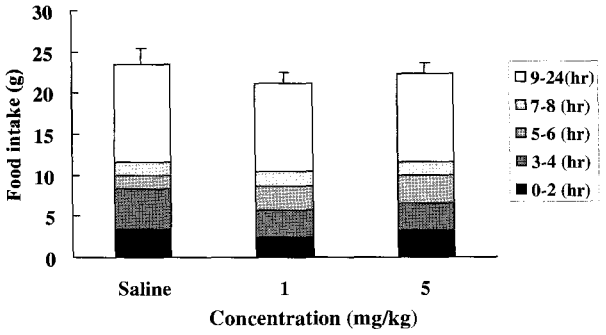

Fig. 6. Effects of intraperitoneal injection of mianserin on food intake. Saline was injected into control rats. There were no statistical differences between the groups during each time interval and 24 hours.

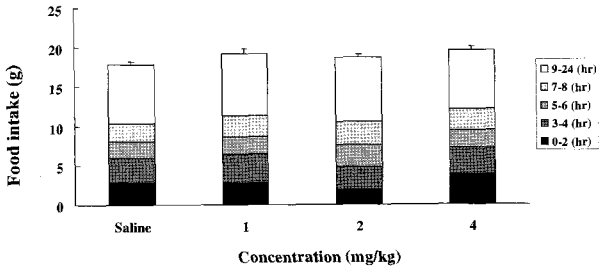

Fig. 7. Effects of intraperitoneal injection of ketanserin on food intake. Saline was injected into control rats. *$p<0.05$ vs control.

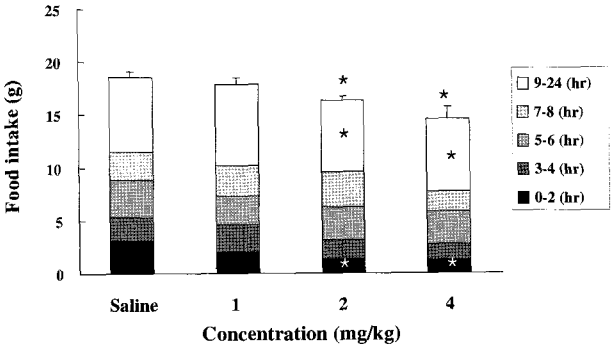

Fig. 8. Effects of intraperitoneal injection of RS 102221 on food intake. Saline was injected into control rats. *$p<0.05$ vs control.

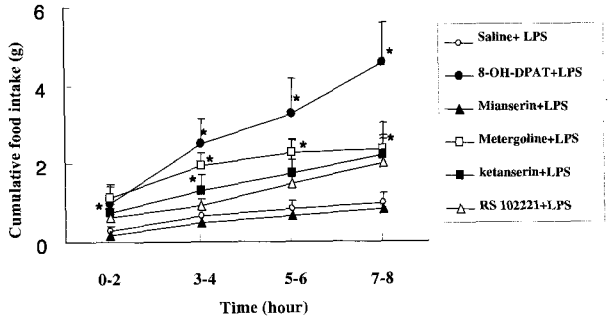

Fig. 9. Effects of various serotonergic drugs on lipopolysaccharide (LPS)-induced anorexia for 8 hours in rats. LPS (500 μg/kg), mianserin (5 mg/kg), metergoline (5 mg/kg), ketanserin (4 mg/kg), and RS 102221 (1 mg/kg) were intraperitoneally injected, and 8-OH-DPAT (1 mg/kg) was subcutaneously injected. Serotonergic drugs were administered 30 minutes before LPS injection. Pretreatment with 8-OH-DPAT, metergoline and ketanserin resulted in the partial reduction of food intake by LPS, but mianserin and RS 102221 exerted no effects on food intake. *$p<0.05$ vs saline + LPS.

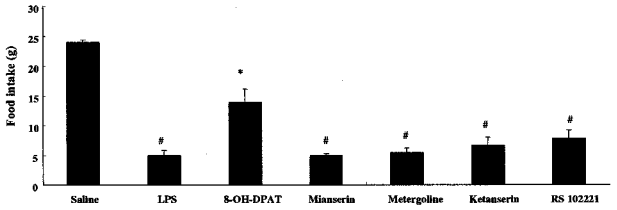

Fig. 10. Effects of various serotonergic drugs on lipopolysaccharide (LPS)-induced anorexia in rats. LPS (500 μg/kg), mianserin (5 mg/kg), metergoline (5 mg/kg), ketanserine (4 mg/kg), and RS 102221 (1 mg/kg) were intraperitoneally injected, and 8-OH-DPAT (1 mg/kg) was subcutaneously injected. Pretreatment with 8-OH-DPAT resulted in a partial recovery of LPS-induced decreased food intake for 24 hours, but the other drugs exerted no effects on food intake for 24 hours. #$p<0.001$ vs saline, *$p<0.05$ vs LPS.

24 hours. Metergoline pretreatment induced elevated cumulative food intake at 2, 4, 6, and 8 hours, but this effect was not detected at 24 hours. Treatment with ketanserin resulted in elevated cumulative food intake, compared to the non-treated group, only at 2 and 4 hours. Mianserin and RS-102221 had no effects on LPS-modulated food intake (Figs. 9 and 10).

DISCUSSION

In the present study, we have demonstrated that the administration of LPS resulted in increased plasma TNF-alpha levels, followed by reduced food intake. LPS-induced anorexia has been suggested to be mediated by an increase in the level of cytokines (McCarthy et al, 1984; Kent S et al, 1992), and the intraperitoneal TNF-alpha injection has been shown to result in reduction of food intake in fasting rats (McCarthy 2000).

LPS administration appears to modulate the levels of energy-regulating amines (Dunn, 1992; Lavicky & Dunn, 1995), and these compounds might constitute targets for the reversal of LPS-induced anorexia. Consistent with this notion, a variety of serotonergic drugs were found to partially ameliorate LPS-induced anorexia in our study. 8-OH-DPAT, a 1A receptor agonist, showed the most profound preventive effect on LPS-induced anorexia . This phenomenon might partially be explained by the fact that, since 1A is an autoreceptor (Bendotti & Samanin, 1986), the stimulation of this receptor inhibits the firing rates of serotonergic neurons, subsequently attenuating serotonin release (Gardier et al, 1996). Therefore, the suppression of serotonergic neural activity by the 1A agonist, 8-OH-DPAT, constitutes a counterbalance of effects of LPS on serotonin metabolism.

The anti-anorexic effects of 8-OH DPAT persisted for 24 hours, whereas metergoline and ketanserin prevented the LPS-induced anorexia for only 8 and 4 hours, respectively. The involvement of serotonin 1B, 2A, and 2C receptors in food intake has been shown in a few previous studies (Sugimoto et al, 1997; Park et al, 1999; Raghavendra & Kulkarni, 2000; Simansky et al, 2002 & 2004). In the previous study, metergoline partially reversed fluoxetine-induced anorexia, although metergoline exerted no immediate intrinsic effects on food intake (Lee & Clifton, 1992). Ketanserin also antagonized the anorexic effects of DL-fenfluramine (Hewson et al, 1988), and the administration of a serotonin analogue induced hypophagia in rats (Simansky et al, 1989). In our study, we also verified the involvement of the serotonin 2A receptor in LPS-induced anorexia. However, since the recovery of anorexia by ketanserin was short-lived, and the 2A/2C receptor antagonist, mianserin, exerted no effects on food intake in the LPS-injected rats, the serotonin 2A receptor might play a marginal role in LPS-induced anorexia. Metergoline was clearly more potent than ketanserin in inhibition of LPS-induced anorexia, therefore, there is a possibility that the serotonin 1B or 2C receptors are also involved in LPS-induced anorexia.

Inconsistent with our results, Hrupka and Langhans (2001) demonstrated that 8-OH-DPAT reduced anorexia for only 2 hours, followed by a compensatory hypophagia. The long-term effects of this drug were not evaluated in their study. They also reported that metergoline and ketanserine had no effect on LPS-induced anorexia. The discrepancy between ours and their results might be attributable to the administration of serotonergic drugs as a pretreatment in our study, whereas Hrupka and Langhans administered the drugs 4 hours after injecting LPS, by which time the cytokines generated by LPS treatment were probably fully active. According to their study, 100 μg/kg LPS, their experimental LPS dose, induced significant levels of cytokines at 90 minutes after injection (Lenczowski et al, 1997). They also measured food intake only for 2 hours, from 4 to 6 hours or from 5 to 7 hours after LPS injection. The results obtained by such a limited measurement schedule might bring in a misinterpretation of the role of serotonergic drugs in LPS-induced anorexia. Another possible reason for the differences might have been in the differences in LPS dosage in the two studies. We used 5 times more LPS than Hrupka and Langhans (Hrupka & Langhans, 2001) did, which would clearly induce more rapid and severe anorexia in our subjects. We strongly belive that the serotonergic system plays a more profound role in more severe cases of anorexia, therefore, inhibition of the serotonergic system might be more effective in cases of severe anorexia.

As for the tested serotonin 2C receptor, RS-102221, we were unable to observe it's function in LPS-induced anorexia. A previous study observe similar finding. However, SB 242084, a serotonin 2C receptor antagonist, exerted a detectable preventive effect on LPS-induced anorexia (von Meyenburg et al, 2003). Therefore, further study might be necessary to exclude the possibility that the 2C receptor is involved in LPS-induced anorexia.

In summary, we found that LPS-induced anorexia could be reversed by inhibition of the serotonergic system, implicating the function of serotonin's function in LPS-induced anorexia. Furthermore, more than one receptor may be involved in this phenomenon, including the serotonin 2A receptor.

ACKNOWLEDGEMENT

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