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A role for serotonin in lipopolysaccharide-induced anorexia in rats

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Abstract

Rats consistently reduce their food intake following injection of bacterial lipopolysaccharides (LPS). Because LPS increases CNS serotonin (5-HT) turnover, and because increases in CNS 5-HT turnover are associated with a decrease in food intake, we conducted a series of studies to examine 5-HT's potential role in LPS-induced anorexia. Chronic CNS 5-HT depletion by cisterna magna (CM) administration of 5,7-dihydroxytryptamine (5,7-DHT) failed to attenuate LPS-induced (100 μg/kg, ip) anorexia. In subsequent experiments, LPS was injected at lights out (hour 0) and [8-hydroxy-2-(di-*n*-propylamino)tetraline (8-OH-DPAT)] or N-CBZ-[(8β)-1,6-dimethylergolin-8-yl]methylamine (metergoline) was injected at hour 5 — the time when LPS-treated rats become anorectic. Food intake was measured during the subsequent 2 h. In LPS-treated rats, 8-OH-DPAT (62.5, 125, or 250 μg/kg, sc) injection increased food intake. In a 2 × 2 factorial arrangement of LPS and 8-OH-DPAT, 125 μg/kg 8-OH-DPAT increased food intake significantly more in LPS-treated rats than in non-LPS-treated rats (significant LPS × 8-OH-DPAT interaction). In LPS-treated rats, 1 and 5 mg/kg metergoline significantly enhanced food intake. However, in a 2 × 2 arrangement of LPS and metergoline, 1 mg/kg metergoline failed to increase food intake in LPS and non-LPS-treated rats in two separate trials. The ability of the 5-HT_{1A} receptor agonist 8-OH-DPAT to attenuate LPS-induced anorexia in rats supports a role of 5-HT in LPS-induced anorexia.

Keywords: Lipopolysaccaride; Food intake; Serotonin; 8-OH-DPAT; Metergoline

1. Introduction

Animals respond to infectious pathogens or injury with a variety of immune, endocrine, metabolic, and behavior responses. These nonspecific symptoms that accompany a pathogenic infection are part of the host's defense reaction, commonly called the 'acute phase response'. Lipopolysaccharides (LPS) are components of the cell wall of gram-negative bacteria, and the major bacterial products responsible for the acute phase response during gram-negative bacterial infections (reviewed in Langhans, 1996). LPS cause myeloid cells to synthesize cytokines such as interleukin-1 (IL-1), interleukin-6, and tumor necrosis factor-α (TNF-α), which then stimulate the various systems responsible for the acute phase response (Hart, 1988). The cytokines IL-1α, IL-1β, and TNF-α potently reduce food intake, and are believed to be the major cytokines responsible for the anorexia that follows administration of LPS in mice and rats (reviewed in Plata Salaman, 1991; Langhans and Hrupka, 1999).

The reductions in food intake that accompany infection must ultimately result from modulation of the CNS mechanisms that control food intake regardless of how cytokines produce the signal that initiates the anorexia. Serotonin is one possible neurotransmitter that may be involved in mediating acute phase response anorexia. Increases in serotonergic activity or stimulation of postsynaptic serotonergic receptors result in a decrease in food intake (Blundell, 1986; Leibowitz, 1986), whereas depleting central 5-HT with specific neurotoxins, diminishing serotonergic transmission by activating 5-HT_{1A} autoreceptors, or blocking postsynaptic 5-HT receptors with specific antagonists often increases food intake (reviewed in Simansky, 1996). It is currently thought that the 5-HT_{1B} and/or 5-HT_{2C} receptors are responsible for mediating many of serotonin's effects on feeding (Bonhaus et al., 1997; Lee and Simansky, 1997; Lucas et al., 1998).

Both peripherally administered LPS (Dunn, 1992a) and IL-1α (Dunn, 1992a,b) activate central serotonergic activity, suggesting a possible role of 5-HT in acute phase response

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anorexia. The changes in serotonergic activity occur in a temporal manner that is consistent with its potential involvement in anorexia (Dunn, 1992a; MohanKumar et al., 1999). Furthermore, these changes occur after low doses of LPS or cytokines, and in hypothalamic areas known to be involved in the control of food intake. For example, peripherally administered LPS (10 μg/kg) increased norepinephrine, dopamine, and 5-HT levels in paraventricular nucleus micropunches (MohanKumar et al., 1999), and 2 ng IL-1α injected directly into the ventromedial hypothalamus of rats caused a transient increase in 5-HT concentration in dialysate in this area and decreased food intake (Yang et al., 1999).

While there is significant correlative evidence for a role of 5-HT in LPS-induced anorexia, to date, there is little causal evidence for such a role of 5-HT. Therefore, we conducted several series of experiments using CNS 5-HT depletion (5,7-DHT), reduction in 5-HT neurotransmission [8-hydroxy-2-(di-*n*-propylamino)tetraline hydrobromide (8-OH-DPAT)] and 5-$HT_{1\ and\ 2}$ receptor antagonism (metergoline) to examine whether these manipulations could attenuate LPS-induced anorexia. Unlike Swiergiel and Dunn (2000), who found no evidence for a role of 5-HT in IL-1-induced anorexia in mice, we report here a potential role for 5-HT in LPS-induced anorexia in the rat.

2. Method

2.1. Animals and housing

Male Sprague–Dawley rats were individually housed in stainless steel hanging wire cages with wire mesh bottoms. Founder rats from Charles River Germany were maintained as a breeding colony under specified pathogen-free conditions in our building in Schwerzenbach, Switzerland. Animal rooms were maintained at 22 ± 0.5°C on a 12:12-h light:dark cycle with lights out at 1000 h. Standard powdered laboratory chow (Nafag, Gossau, Switzerland) and water were available ad libitum. All procedures were approved by the Kanton of Zurich's Animal Use and Care Committee.

2.2. Experiment 1: the role of chronic CNS 5-HT depletion on LPS-induced anorexia

Experiment 1 was conducted to determine whether chronic CNS 5-HT depletion using 5,7-dihydroxytryptamine (5,7-DHT) would attenuate LPS-induced anorexia. Thirty-five rats initially weighing 316 ± 4 g (mean ± S.E.M.) were ranked according to body weight (BW), and were randomly assigned to cisterna magna (CM) injection of either vehicle or 5,7-DHT. Within 30–50 min before 5,7-DHT administration, rats received an intraperitoneal injection of desmethylimipramine (Desipramine, Sigma, St Louis, MO; 25 mg/kg BW) to render 5,7-DHT uptake more specific to 5-HT neurons. For CM injections, rats were lightly anesthetized with methoxyflurane (Metofane, Pittman Moore, Washington Crossing, NJ) placed in a stereotaxic apparatus, and the head was flexed downward so that a 28-gauge needle could be lowered between the cervical vertebrae and into the CM. The needle was connected to a 25-μl Hamilton syringe with a clear Tygon tube 8 cm in length. Needle placement was verified by drawing a small amount of cerebrospinal fluid into the injection needle, and observing the clear liquid in the tubing. Rats received a 20-μl injection of 5,7-DHT (200 μg free base, 419 μg 5,7-DHT creatinine sulfate salt, Sigma, St. Louis, MO; D-0136) or vehicle (1% ascorbic acid in sterile deionized water). Anesthesia was maintained throughout the procedure via a nose cone containing methoxyflurane. Following 5,7-DHT administration, rats received sodium pentobarbital injections (30 mg/kg BW + supplemental injections when necessary; Vetanorcol, Veterinaria, Zurich) to retard the development of seizures, and were placed on a heating pad until they regained consciousness, after which they were returned to their home cage.

Nineteen days after 5,7-DHT treatment, rats were injected with 100 μg/kg BW LPS or saline in a 2 × 2 factorial arrangement of LPS and 5,7-DHT treatment. Seven days after the first trial, rats were crossed over (within the control/5,7-DHT group) so that all rats received LPS injections. Rats were tested at this point in time based on results from Coscina and de Rooy (1992). LPS was injected intraperitoneally just prior to lights out. Food intake was recorded at 3, 6, 9, 12, and 24 h after lights out. Food intake was measured as the difference in food cup weights after correcting for spillage. A 40-W red light was left on at all times to help facilitate food intake measurements during the dark phase.

At the conclusion of the study, rats were killed by decapitation, and brains were rapidly removed (within 60 s) for determination of 5-HT levels. The brains were frozen on dry ice and stored at −80°C until 5-HT determination. The cortex, hypothalamus, and brainstems were dissected, weighed, and homogenized in 0.1% perchloric acid containing isoproterenol, as an internal standard. Samples were centrifuged at 15,000 × *g* for 15 min in a microcentrifuge in a cold room. Samples were filtered through at 0.45-μm filter, and 5-μl aliquots were injected onto a Chrompack glass column [100 (L) × 3 (ID) × 9 (OD) mm, 5 μm silica gel] for chromatographic separations. The mobile phase (flow rate = 0.5 ml/min) consisted of 20 mM KH_2PO_4, 1.5–2 mM octanesulfonic acid, and 5% (v/v) acetonitrile (pH = 3.0). Detection was done with an electrochemical amperometric detector (Model LC-4B, Bioanalytical Systems, West Lafayette, IN, USA) with a working electrode set at +750 mV vs. Ag/AgCl. The position and height of the peaks of the endogenous components were compared with external calibrating standard solutions for 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), and the internal standard, isoproterenol.

2.3. Experiment 2, Trial 1: dose–response curve to 8-OH-DPAT in LPS-treated rats

Because activation of the 5-HT_{1A} autoreceptor receptor using the agonist 8-OH-DPAT decreases 5-HTergic transmission in the brain and increases food intake under certain conditions, we tested the ability of 8-OH-DPAT to increase food intake in LPS-treated rats. Twenty-four rats (584 ± 8 g BW, mean ± S.E.M.) were housed in individual cages equipped for computerized monitoring of feeding behavior. Cages ($38 \times 23 \times 13$ cm, $L \times W \times H$) were constructed of plastic, and contained a wire mesh bottom. A 6.5-cm diameter tube extended 20 cm from the side of the cage, and then downward 4 cm to provide access to a spill-resistant food cup situated on a Mettler PM300 balance. Meal pattern data were collected using an Olivetti M240 computer that monitored the balances' output approximately every 30 s. Access to the food cup could be denied by closing a chute built into the tube extending horizontally from the cage.

On the day of the experiment, food cups were filled 1 h before lights out, and food access was denied by closing the feeding chute. All rats received intraperitoneal injections of LPS (100 μg/kg BW) within 15 min prior to lights out. At lights out, the feeding chute was opened, and rats were left undisturbed until 4 h after lights out, at which time food intake was recorded and food access was denied. Drug was then prepared, and within 15 min before 5 h (a time when LPS-injected rats eat very little), rats received subcutaneous injections of either 0, 62.5, 125, or 250 μg/kg BW 8-OH-DPAT (Sigma; H8520) dissolved in saline. At 5 h, feeding chutes were opened and rats had free access to food. Food intake and meal parameters were assessed from 5 to 7 h, the period when 8-OH-DPAT is effective at enhancing feeding.

2.4. Experiment 2, Trial 2: 2×2 factorial arrangement — 8-OH-DPAT and LPS

To access the ability of 8-OH-DPAT to enhance food intake under control vs. LPS-induced anorexia, a 2×2 factorial design was conducted using LPS vs. saline treatment and 8-OH-DPAT (125 μg/kg BW) vs. vehicle as factors. Rats ($N = 28$) weighing 390 ± 4 g (mean ± S.E.M.) were blocked according to BW and randomly assigned to treatment groups. As described above and in Fig. 1, 15 min before lights out, half the rats received LPS injections (100 μg/kg BW, ip) while the other half received saline. Food cups, which had been removed 1.5 h before lights out, were replaced into the cage at lights out. Food was recorded at 2 and 4 h after lights out. During the 4-h food intake measurement, food cups were removed from the cage. 8-OH-DPAT was then prepared, and starting 15 min before 5 h, half the rats in each group (LPS vs. vehicle) received subcutaneous injections of either 8-OH-DPAT or saline. At 5 h, food cups were returned to the cage and food intake was recorded again at 7 and 9 h.

Fig. 1. Protocol for drug and LPS administration. Fifteen minutes before lights out, half the rats received LPS injections (100 μg/kg BW, ip) while the other half received saline. Food cups were placed into the cage at lights out (0 h). Food was recorded at 2 and 4 h after lights out. During the 4-h food-intake measurement, food cups were removed from the cage. Starting 15 min before 5 h, half the rats in each group (LPS vs. vehicle) received subcutaneous injections of either drug or vehicle. At 5 h, food cups were returned to the cage and food intake was recorded again at 7 and 9 h.

2.5. Experiment 3, Trial 1: dose–response curve to metergoline in LPS-treated rats

Experiment 3 was conducted to assess the ability of N-CBZ-[(8β)-1,6-dimethylergolin-8-yl]methylamine (metergoline) to attenuate LPS-induced anorexia. Rats ($N = 28$, 355 ± 5 g, mean ± S.E.M.) were blocked according to BW and randomly assigned to one of four treatment groups. Treatments included control (1% ascorbic acid), 0.25, 1.0, or 5.0 mg/kg ip metergoline (Sigma; M3668). The vehicle had been found in a preliminary study to not affect food intake under our test conditions (i.e., it did not alter 5–7 h food intake). As described in Experiment 2, rats were injected with LPS (100 μg/kg BW, ip) within 15 min of lights out, and then with metergoline within 15 min before 5 h. Eight days after the trial, a second experiment was conducted to determine whether these doses of metergoline alone altered food intake using the same experimental protocol, except that rats did not receive LPS pretreatment. All rats (380 ± 5 g BW, mean ± S.E.M.) received saline injections prior to lights out (instead of LPS), and within 15 min before 5 h, rats received the same doses of metergoline in a counterbalanced order.

2.6. Experiment 3, Trial 2: 2×2 factorial arrangement — LPS and metergoline

Because the 1 mg/kg BW metergoline dose significantly increased food intake after LPS administration, but did not alter food intake after saline pretreatment, this dose was chosen to compare the effect of LPS vs. metergoline in a 2×2 factorial arrangement of treatments. Rats ($N = 28$, 321 ± 3 g BW, mean ± S.E.M.) were blocked according to BW and randomly assigned to treatment groups. Using the protocol described in Fig. 1, half the rats received LPS (100 μg/kg BW) while the others received saline. At 5 h, half the rats in each group received metergoline (1 mg/kg BW in 1% ascorbic acid, ip) or vehicle.

2.7. Statistical analysis

Because 5,7-DHT treatment did not reduce brain 5-HT in all rats, only rats that showed significant (>65%) cortex 5-HT depletion were included in the statistical analysis. This included eight 5,7-DHT-treated rats. Among control rats, two completely failed to reduce food intake after LPS treatment, and were removed as outliers so that 12 were included in the statistical analysis. Because animals were crossed over so that all received LPS treatment, data were analyzed as a split-plot ANOVA using General Linear Models (GLM) procedures (SAS, SAS Institute, Carey, NC, Release 6.12). 5,7-DHT treatment and carryover effects were included in the main plot, and between subjects residuals were used as the error term for determining 5,7-DHT effects. The effect of LPS treatment and day of injection were included in the subplot. The within-subjects residual was used as the error term for LPS treatment effects. Because this model cannot analyze for 5,7-DHT × LPS interactions, the difference in food intake between saline treatment and LPS treatment for each animal was analyzed by ANOVA. A significant 5,7-DHT treatment effect was taken to indicate a significant interaction. Results from dose–response trials were analyzed using GLM procedures appropriate for a one-way ANOVA with blocking. When an overall significant ANOVA occurred, treatment means were compared using Duncan's multiple range test. Results from LPS × Drug interaction trials were analyzed using GLM procedures appropriate for a 2 × 2 factorial arrangement of LPS and drug in a randomized complete block design. In all experiments, blocks consisted of rats with similar BWs. Results are expressed as mean ± S.E.M.

3. Results

3.1. Experiment 1: the role of chronic CNS 5-HT depletion on LPS-induced anorexia

5,7-DHT treatment resulted in an 80% reduction in cortex 5-HT (256.2 ± 24.7 vs. 48.6 ± 6.6 ng/g for control and 5,7-DHT-treated rats, respectively). Hypothalamic 5-HT was much less dramatically affected by 5,7-DHT treatment (30% reduction). Hypothalamic 5-HT levels were 812 ± 63 vs. 570 ± 45 ng/g for control and 5,7-DHT-treated rats, respectively. Brainstem 5-HT levels were 599 ± 28 vs. 501 ± 27 ng/g for control and 5,7-DHT-treated rats, respectively. Changes in 5-HIAA levels after 5,7-DHT treatment were similar to those of 5-HT (65% reduction in cortex, 26% reduction in hypothalamus, 41% reduction in brainstem).

Baseline food intake was similar between 5,7-DHT-treated rats and controls. Initial BW prior to 5,7-DHT treatment was 312 ± 6 and 310 ± 10 g for control and 5,7-DHT-treated rats, respectively. Prior to the second trial, control and 5,7-DHT-treated rats weighed 378 ± 10 and 361 ± 13 g, respectively, so that BW gain between controls and 5,7-DHT-treated rats (66 ± 5 and 51 ± 4 g, respectively) was significantly less in 5,7-DHT-treated rats [$F(1,17) = 4.83$, $P < .05$]. Baseline food intake for the 2 days prior to the second trial was not significantly different between treatment groups (19.9 ± 0.7 vs. 21.1 ± 0.7 g/day for control and 5,7-DHT-treated rats, respectively; $F(1,17) = 1.57$, $P > .2$).

From 3 h after lights out onward, LPS-treated rats ate significantly less than non-LPS-treated rats [$F(1,17)$ all >22.3, all $P < .001$; Fig. 2] while 5,7-DHT-treated rats ate similar amounts of food as control rats [$F(1,18)$ all <2.24, all $P > .15$]. No statistically significant LPS × 5,7-DHT interaction occurred at any time point [$F(1,17)$ all <0.46, all $P > .5$], meaning that both control and 5,7-DHT-treated rats reduced their food intake similarly after LPS treatment.

3.2. Experiment 2, Trial 1: dose–response curve to 8-OH-DPAT in LPS-treated rats

LPS-treated rats ate significantly more food during the 2 h following all doses of 8-OH-DPAT [$F(3,20) = 8.86$, $P < .001$, Fig. 3]. During this time, controls that received LPS alone ate 1.7 ± 0.4 g, while all other groups consumed approximately 5 g, a 3-g increase in food intake compared to controls. Meal pattern analysis was also conducted during the 2 h following 8-OH-DPAT. Meal size was 2.1 ± 0.3, 3.8 ± 0.6, 3.3 ± 0.5, and 3.2 ± 0.7 g for control, 62.5, 125, and 250 μg/kg 8-OH-DPAT. Although rats receiving 8-OH-DPAT consistently ate larger meals than control rats, the overall ANOVA was not significant [$F(3,19) = 1.68$, $P > .2$]. Meal duration was 11 ± 4, 20 ± 3, 24 ± 6, and 25 ± 4 min for control, 62.5, 125, and 250 μg/kg 8-OH-DPAT, respectively. Again, although meal duration was twice as long in 8-OH-DPAT-treated rats, because of variability, the overall ANOVA was not statistically different [$F(3,19) = 1.83$, $P > .15$].

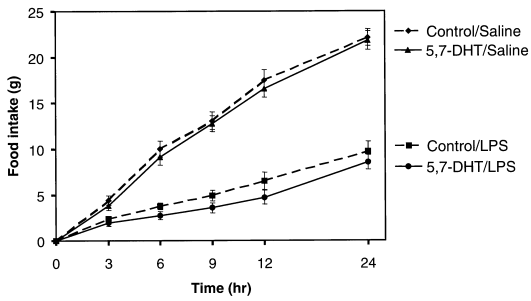

Fig. 2. Food intake of 5,7-DHT-treated rats following LPS administration. Rats were chronically CNS 5-HT depleted using CM administration of 5,7-DHT (200 μg free base). Twenty-six days after 5,7-DHT treatment, rats were injected with 100 μg/kg BW LPS or saline just prior to lights out. Data are means ± S.E.M. of food intake at the times indicated. From 3 h after lights out onward, LPS-treated rats ate significantly less than non-LPS (saline)-treated rats (all $P < .05$). 5,7-DHT-treated rats ate similar amounts of food as non-5,7-DHT-treated rats regardless of whether they were injected with LPS or saline.

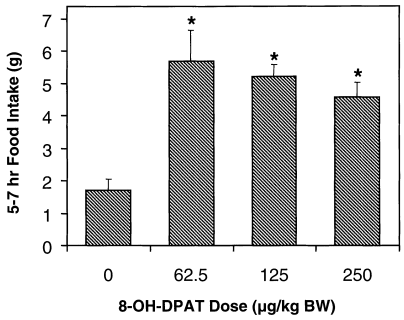

Fig. 3. Dose–response curve to 8-OH-DPAT in LPS-treated rats. Twenty-four rats were treated with LPS before lights out as described in Fig. 1. Within 15 min before 5 h, rats received subcutaneous injections of either 0, 62.5, 125, or 250 μg/kg BW 8-OH-DPAT. Data are means ± S.E.M. of food intake for the 2 h following 8-OH-DPAT administration, the period when 8-OH-DPAT is effective at enhancing feeding. Rats ate significantly more food during the 2 h following all doses of 8-OH-DPAT ($P < .001$).

3.3. Experiment 2, Trial 2: 2×2 factorial arrangement — 8-OH-DPAT and LPS

Results from this experiment are presented in Fig. 4 and Table 1. As expected, LPS-treated rats ate significantly less than control rats during 5–7 h [$F(1,24) = 74$, $P < .0001$]. 8-OH-DPAT-treated rats ate significantly more during 5–7 h [$F(1,24) = 14.9$, $P < .001$], but this difference was due primarily to an increase in food intake of LPS/8-OH-DPAT-treated rats as noted by a significant LPS × 8-OH-DPAT interaction [$F(1,24) = 4.34$, $P < .05$]. That is, although 8-OH-DPAT increased food intake slightly under non-LPS conditions (0.6 g), it increased food intake to a greater extent (2 g) in LPS-injected rats.

From 7 to 9 h, 8-OH-DPAT-treated rats actually ate significantly less than non-8-OH-DPAT-treated rats [$F(1,24) = 8.64$, $P < .01$] so that 8-OH-DPAT was no longer effective at stimulating food intake during this period (Table 1). Although there was no LPS × 8-OH-DPAT interaction,

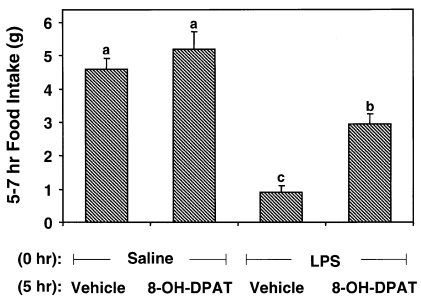

Fig. 4. Response of LPS-treated and control rats to 8-OH-DPAT. LPS-treated (100 μg/kg BW, ip) and control rats were injected with 8-OH-DPAT (125 μg/kg) or vehicle as described in Fig. 1. Data are means ± S.E.M. of food intake of the 2 h following 8-OH-DPAT administration. LPS-treated rats ate less than control rats ($P < .0001$) and 8-OH-DPAT-treated rats ate more than vehicle-treated rats ($P < .001$). This difference was due primarily to an increase in food intake of LPS/8-OH-DPAT-treated rats (LPS × 8-OH-DPAT interaction, $P < .05$).

Table 1
Food intake[a] (g) of rats injected with LPS (100 μg/kg BW, ip) or saline at dark onset, and 8-OH-DPAT (125 μg/kg BW, sc) or vehicle 5 h later — a time when LPS-treated rats are anorectic (Experiment 2, Trial 2)

	Saline		LPS		
Time after LPS (h)	Vehicle	8-OH-DPAT	Vehicle	8-OH-DPAT	Pooled S.E.M.
5–7[b]	4.6	5.2	0.9	3.0	0.3
7–9[c]	2.0	1.3	1.5	0.3	0.2
5–9[d]	6.6	6.5	2.4	3.3	0.7

[a] Values represent means of seven rats/treatment.
[b] Effect of LPS, $P < .0001$; 8-OH-DPAT, $P < .001$; LPS × 8-OH-DPAT, $P < .05$.
[c] Effect of LPS, $P < .02$; 8-OH-DPAT, $P < .01$; LPS × 8-OH-DPAT, $P > .4$.
[d] Effect of LPS, $P < .0001$; 8-OH-DPAT, $P > .3$; LPS × 8-OH-DPAT, $P > .3$.

the hypophagia was slightly more pronounced in LPS/8-OH-DPAT-treated rats than saline/8-OH-DPAT-treated rats. Consequently, from 5 to 9 h, there was no overall effect of 8-OH-DPAT on food intake [$F(1,24) = 0.79$, $P > .35$], and LPS-treated rats ate similar amounts of food regardless of whether they received 8-OH-DPAT or saline (3.3 ± 0.5 vs. 2.4 ± 0.5 g, respectively). Likewise, saline (non-LPS-treated) rats ate the same amount of food from 5 to 9 h regardless of whether they received 8-OH-DPAT or saline (6.56 ± 0.6 vs. 6.60 ± 0.4 g, respectively).

3.4. Experiment 3, Trial 1: dose–response curve to metergoline in LPS-treated rats

Metergoline administration resulted in a dose-dependent increase in 5–7 h food intake in LPS-treated rats [$F(3,24) = 4.49$, $P < .02$; Fig. 5]. Five to seven hours food intake was 0.2 ± 0.1, 1.3 ± 0.6, 1.8 ± 0.4, and 2.2 ± 0.3 for

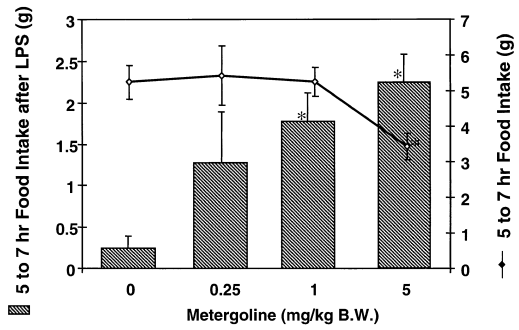

Fig. 5. Dose–response curve to metergoline in LPS-treated rats. Twenty-eight rats were treated with LPS before lights out as described in Fig. 1. Within 15 min before 5 h, rats received subcutaneous injections of either 0, 0.025, 1, or 5 mg/kg BW metergoline. Slashed bars represent means ± S.E.M. of food intake for the 2 h following metergoline administration. Metergoline administration resulted in a dose-dependent increase in 5–7 h food intake in LPS-treated rats ($P < .02$). Both the 1- and 5-mg/kg BW doses of metergoline resulted in 5–7 h food intakes that were statistically higher than controls. When metergoline was administered without prior LPS administration (lines/triangles), the highest dose (5 mg/kg) resulted in a significant reduction in 5–7 h food intake ($P < .05$).

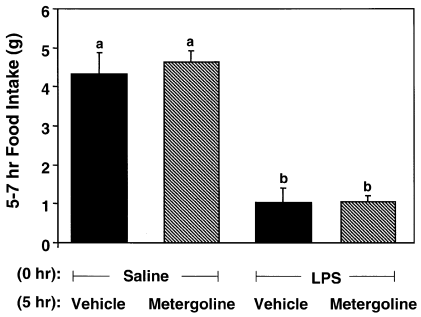

Fig. 6. Response of control and LPS-treated rats to metergoline. LPS-treated (100 μg/kg BW, ip) and control rats were injected with metergoline (1 mg/kg BW, ip) or vehicle as described in Fig. 1. Data are means ± S.E.M. of food intake for the 2 h following metergoline administration. From 5 to 7 h, LPS-treated rats ate less than control rats ($P < .0001$); however, metergoline treatment had no effect on food intake ($P > .6$).

control, 0.25, 1, and 5 mg/kg metergoline, respectively. Both the 1- and 5-mg/kg doses of metergoline resulted in 5–7 h food intakes that were statistically higher than controls. When metergoline was administered without prior LPS administration, the highest dose (5 mg/kg BW) resulted in a significant reduction in 5–7 h food intake [$F(3,24) = 3.03$, $P < .05$]. Five to seven hours food intake was 5.2 ± 0.5, 5.4 ± 0.8, 5.3 ± 0.4, and 3.4 ± 0.4 g for control, 0.25, 1, and 5 mg/kg BW, respectively.

3.5. Experiment 3, Trial 2: 2×2 factorial arrangement — LPS and metergoline

Because 1 mg/kg BW significantly increased food intake after LPS administration, but did not alter food intake after saline pretreatment, this dose was chosen to compare the effect of LPS and metergoline in a 2 × 2 factorial arrangement of LPS vs. saline and metergoline vs. vehicle (Fig. 6). As expected, LPS treatment significantly reduced 5–7 h food intake [$F(1,24) = 83.2$, $P < .0001$]; however, 1 mg/kg BW metergoline had no effect on food intake [$F(1,24) = 0.21$, $P > .6$] in both this trial and in a second separate trial (results not presented).

4. Discussion

We provide evidence for a possible role of the 5-HT system in LPS-induced anorexia. Inhibition of 5-HT transmission via 8-OH-DPAT (5-HT_{1A} receptor agonist) consistently enhanced food intake, and did so significantly more in LPS-treated rats than controls. Numerous pharmacological studies support an inhibitory role of 5-HT in the control of feeding. Our results are consistent with the hypothesis that the 5-HT system is also involved in the inhibition of feeding under pathophysiological conditions, and that reducing 5-HT activity can restore feeding, at least transiently.

In Experiment 1, CM administration of 5,7-DHT did not alter food intake after LPS administration. However, these results are consistent with other reports, in which CM or direct raphe nucleus injection of 5,7-DHT did not cause chronic overeating or BW gain in rats (Coscina and de Rooy, 1992; Hoebel et al., 1978; Currie et al., 1998). Whereas 5,7-DHT treatment in Experiment 1 significantly reduced cortical 5-HT and 5-HIAA levels, it was much less effective at reducing hypothalamic or brainstem 5-HT and 5-HIAA levels. This is consistent with results from Saller and Stricker (1976), who also found a much greater effect of intracerebroventricular 5,7-DHT on telencephalon 5-HT depletion than on diencephalon or brainstem 5-HT depletion. The inability of 5,7-DHT treatment to adequately deplete hypothalamic or brainstem 5-HT levels might explain the lack of an effect of 5,7-DHT on LPS-induced anorexia. Another possibility is that a change in the functional status of the remaining serotonergic neurons is sufficient to mediate the anorexic effect of LPS (Kirby et al., 1995). While it is believed that 5-HT may partially mediate cytokine (IL-1β and TNF-α) effects during cancer anorexia (Meguid et al., 1992; Chance et al., 1983a), CM 5,7-DHT treatment did not affect the rats' anorectic response to cancer (Chance et al., 1983b). Intracerebroventricular 5,7-DHT also did not alter the mice's anorectic response to IL-1 (Swiergiel and Dunn, 2000). Thus, our feeding and neurochemical results are clearly not inconsistent with other published reports using 5,7-DHT in models of food intake and/or anorexia.

The main finding of this paper is that the 5-HT_{1A} autoreceptor agonist 8-OH-DPAT consistently increased short-term food intake in LPS-treated rats. Furthermore, 8-OH-DPAT increased food intake more in LPS-treated rats than in non-LPS-treated rats. 5-HT_{1A} agonists such as 8-OH-DPAT, ipsapirone, buspirone, gepirone, and LY-165-163, increase food intake in rats under certain conditions (Dourish et al., 1985; Gilbert and Dourish, 1987; Hutson et al., 1988a). In the brain, 5-HT_{1A} receptors are mainly localized in the dorsal and medial raphe nuclei (which contain serotonergic cell bodies), as well as in numerous areas of the limbic system. In the raphe nucleus, 5-HT_{1A} receptors are presynaptic autoreceptors that regulate 5-HT neuronal activity and 5-HT synthesis and release, both locally and in terminal regions (reviewed in Zifa and Fillion, 1992). 8-OH-DPAT acts as a full agonist at raphe 5-HT_{1A} receptors, and it is believed to act here to enhance food intake, because 8-OH-DPAT injection into either the dorsal or medial raphe nuclei stimulates feeding (Currie and Coscina, 1993; Fletcher, 1991). The doses of 8-OH-DPAT used in these experiments were within the range typically used to stimulate feeding in rats, but were significantly below those which produce the 5-HT "syndrome" (0.5 to 2 mg/kg BW, sc) (hyperlocomotion, head weaving, reciprocal forepaw treading, and flat body posture; Tricklebank et al., 1984).

It must be noted that even though 8-OH-DPAT enhanced food intake for 2 h post-injection, a compensatory hypophagia occurred from 2 to 4 h post-injection so

that overall food intake was not affected by 8-OH-DPAT treatment. It appears that other compensatory mechanisms also come into play. It would be interesting to determine whether longer-term administration of 8-OH-DPAT would prolong the enhanced eating effect. Unfortunately, with respect to feeding, 5-HT_{1A} agonist administration leads to rapid tolerance development, presumably due to the desensitization of the autoreceptors (Kennett et al., 1987). That is, a single injection of 8-OH-DPAT or ipsapirone attenuates the hyperphagic response to subsequent injection on the following day or 5 days later (Kennett et al., 1987).

It is interesting that metergoline was not consistently effective at increasing food intake after LPS. In the initial dose–response trial, both 1 and 5 mg/kg BW metergoline did increase food intake above control levels; however, this seems to have been due to the low food intake of controls rather than a strong feeding stimulatory effect of metergoline treatment. Metergoline has been reported to increase food intake under certain conditions, such as in satiated rats (Fletcher, 1988), and during a 4-h light phase food-intake trial (Dourish et al., 1989). In addition, the feeding suppressive effects of fenfluramine (Neill and Cooper, 1989) and fluoxetine (Lee and Clifton, 1992) have been blocked or attenuated by metergoline. Because metergoline can also block 8-OH-DPAT-induced feeding (Hutson et al., 1988a), it is possible that its antagonist, rather than agonist, action at the 5-HT_{1A} receptor explains its lack of effect on LPS-induced anorexia.

It is currently thought that the 5-HT_{1B} and/or 5-HT_{2C} receptors are responsible for mediating many of serotonin's effects on food intake. The anorectic effect of D-fenfluramine is absent in 5-HT_{1B} knock-out mice (Lucas et al., 1998), and is reversible by 5-HT_{1B} and 5-$HT_{2A/2C}$ receptor antagonism (Lee and Simansky, 1997). On a more long-term basis, daily 5-HT_{2C} receptor antagonist injection was reported to enhance the rats' food intake and weight gain in one study (Bonhaus et al., 1997) but not in another (Kennett et al., 1997). It remains to be determined whether selective 5-HT receptor antagonists will attenuate LPS-induced anorexia.

It is unclear exactly where 5-HT may be mediating LPS's anorexic effect. In the brain, the paraventricular nucleus and ventromedial hypothalamus are clearly candidate areas based on increases in serotonergic activity in these areas following injections of LPS (MohanKumar et al., 1999) or IL-1α (Yang et al., 1999). Injections of 5-HT_{1B} agonists into the parabrachial nucleus (Lee et al., 1998) or the paraventricular nucleus (Hutson et al., 1988b) reduced food intake in rats. With respect to 5-HT_{1A} receptors, flesinoxan (a 5-HT_{1A} agonist) and p-CPA increased PVN arcuate NPY protein levels along with food intake in rats (Dryden et al., 1996). Outside the CNS, 5-HT_{1A} receptors exist on the enteric nervous system, especially around the bowel and pancreas (Kirchgessner et al., 1996), and are involved in mediating synaptic transmission (Gershon, 1991), but their physiological role is not clear.

Even though 5-HT blockade partially attenuated LPS-induced anorexia, it did not completely restore feeding, suggesting that other neurochemical mechanisms are also involved in LPS-induced anorexia. These may include neurotransmitters such as norepinephrine or dopamine (MohanKumar et al., 1999) or neuropeptides such as neuropeptide Y (Sonti et al., 1996) and α-melanocyte stimulating hormone (Huang et al., 1999). Nonetheless, the results of this study do support a role for 5-HT in LPS-induced anorexia in the rat. Whether this response is mediated specifically via the 5-HT_{1A} receptor or other 5-HT receptors awaits further investigation.

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