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Evidence that the anorexia induced by lipopolysaccharide is mediated by the 5-HT_{2C} receptor

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Abstract

Rats consistently reduce their food intake following injections of bacterial lipopolysaccharides (LPS). Because inhibition of serotonergic (5-HT) activity by 8-OH-DPAT (5-HT_{1A} activation) attenuates LPS-induced anorexia, we conducted a series of studies to examine whether other 5-HT-receptors are involved in the mediation of peripheral LPS-induced anorexia. In all experiments, rats were injected with LPS (100 μg/kg body weight [BW] ip) at lights out (hour 0). Antagonists were administered peripherally at hour 4, shortly after the onset of anorexia, which presumably follows the enhanced cytokine production after LPS. Food intake was then recorded during the subsequent 2 h or longer. 5-HT receptor antagonists cyanopindolol and SB 224289 (5-HT_{1B}), ketanserin (5-HT_{2A}), RS-102221 (5-HT_{2C}), and metoclopramide (5-HT_3) failed to attenuate LPS-induced anorexia. In contrast, both ritanserin (5-$HT_{2A/C}$-receptor antagonist) (0.5 mg/kg BW) and SB 242084 (5-HT_{2C}) (0.3 mg/kg BW) attenuated LPS-induced anorexia at doses that did not alter food intake in non-LPS-treated rats (all $P<.01$). Our results suggest that at least part of the anorexia following peripheral LPS administration is mediated through an enhanced 5-HT-ergic activity and the 5-HT_{2C} receptor.

Keywords: Lipopolysaccharide; Anorexia; Food intake; Serotonin; SB 242084; Ritanserin

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' (APR). The APR including its behavioral changes, such as the concomitant anorexia, are thought to be mediated by proinflammatory cytokines (e.g. interferon-γ [IFN-γ], interleukin-1β [IL-1β], tumor necrosis factor-α [TNFα]). Lipopolysaccharides (LPS) are an integral component of the outer membrane of most gram-negative bacteria that are released during bacterial lysis or proliferation (Rietschel et al., 1994). LPS stimulates immune cells to produce proinflammatory cytokines, and its exogenous administration is used as an animal model of acute gram-negative bacterial infection (Tilders et al., 1994).

LPS or cytokine administration is known to cause changes in the CNS concentrations of many neuropeptides and neurotransmitters including serotonin (5-HT). 5-HT has often been shown to inhibit feeding (Blundell, 1984), and it may also do so during the APR because inhibiting 5-HT-ergic activity with the 5-HT_{1A} autoreceptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetraline (8-OH-DPAT) attenuated the anorexia induced by intraperitoneally injected LPS (Hrupka and Langhans, 2001). However, it is not known if a specific postsynaptic 5-HT receptor mediates the anorectic response to LPS or proinflammatory cytokines.

Although fourteen 5-HT receptor subtypes have been identified (Hoyer et al., 1994), current studies mainly implicate the 5-HT_{2C} and/or the 5-HT_{1B} receptors as mediators of the anorectic action of 5-HT. Agonists to these receptors reduce food intake (Clifton, 2000; Clifton et al., 2000; Vickers et al., 2000) and the anorectic effect of D-fenfluramine is blocked or attenuated by 5-HT_{1B} or 5-HT_{2C}

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receptor antagonists (Neill and Cooper, 1989; Vickers et al., 2001) or in mice genetically deficient in these receptors (Lucas et al., 1998; Vickers et al., 1999). 5-HT_{2C} receptor knockout mice are also obese compared to wild-type mice (Tecott et al., 1995).

Nonetheless, other 5-HT receptor subtypes have also been implicated in the control of feeding. For example, 5-HT_{2A} receptor activation attenuates neuropeptide Y-stimulated feeding (Currie and Coscina, 1998), which may be important for the APR because intracerebroventricular administration of neuropeptide Y attenuates IL-1β-induced anorexia (Sonti et al., 1996). The 5-HT_3 receptor may also participate in the control of feeding under certain conditions such as during consumption of amino acid-imbalanced diets (Hrupka et al., 1991).

Because of the potential role of the 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_3 receptors in the control of feeding, we employed pharmacological antagonists to examine the role of these receptors in LPS-induced anorexia. Our results suggest that the 5-HT_{2C} receptor is the main 5-HT-ergic receptor involved in LPS-induced anorexia.

2. Methods

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. Injection protocol 1: dose–response and 2×2 factorial arrangement

Food cups were removed from the cages 1 h prior to lights out, filled with food and weighed. All rats received intraperitoneal injections (100 μg/kg body weight [BW]) of LPS (from *Escherichia coli*, serotype 0111:B4, No. L-2630, Sigma) within 15 min prior to lights out. After the injections, food cups were returned to the cages and rats were left undisturbed until 3 h after lights out, at which time food intake was recorded and food access was denied. Food intake was measured as the difference in food cup weights after correcting for spillage. During 0–3 h, rats ate an average of 4–4.5 g regardless of LPS treatment in all trials. This is because it requires some time for the LPS to stimulate cytokine production, and for the cytokines to trigger the anorectic response. Therefore, these feeding data are not presented in the Results section. The antagonist being tested was prepared, and administered within 15 min before 4 h (a time shortly after the anorexia begins in LPS-injected in rats). Food intake was assessed from 4 to 6 h after LPS injection, and in some experiments also 6–9 h after LPS. This experimental paradigm was chosen to synchronize the time of action of the 5-HT antagonists with the onset of anorexia after LPS injection.

In all studies, rats were randomly assigned to treatments within blocks. In the dose–response studies, all rats were injected with LPS. One group of rats was then treated with vehicle and the other rats were treated with different doses of the particular drug. In studies using a 2 × 2 factorial arrangement of LPS and drug, half the rats in each group (LPS vs. vehicle) received injections of either drug or vehicle.

2.3. Experiment 1: dose–response curve to cyanopindolol and SB 224289 in LPS-treated rats

We used 4-(3-[(1,1-dimethylethyl)amino]-2hydroxypropyl)1*H*-indole-2-carbonitrile, cyanopindolol (Sigma, St. Louis, MO, USA, Cat. No. C-238) and 1′-methyl-5-([2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-biphenyl-4-yl]carbonyl)-2,3,6,7-tetrahydrospiro(furol[2,3-f]indole-3,4′-piperidine), SB 224289 (Sigma, Cat. No. S-201) to test the role of the 5-HT_{1B} receptors in LPS-induced anorexia. In the cyanopindolol trial, 28 rats (323 ± 4 g BW [mean ± S.E.M.]) were used. Rats received subcutaneous injections of 0.3 or 0.6 mg/kg BW cyanopindolol dissolved in distilled water (Neill and Cooper, 1989). SB 224289 was tested in 28 other rats (263 ± 3 g BW). They received intraperitoneal injections of 0.05, 0.5, or 5 mg/kg BW SB 224289 dissolved in distilled water with the aid of brief sonication and warming on a hot plate (Vickers et al., 2001). In both trials, food intake was recorded as described above.

2.4. Experiment 2, Trial 1: dose–response curve to ritanserin in LPS-treated rats

To test whether LPS-induced anorexia is mediated through the 5-HT_2 receptors, we used the 5-$HT_{2A/C}$ receptor antagonist 6-(2-[4-(bis[4-fluorophenyl]methylene)-1-piperidinyl]ethyl)-7-methyl-5*H*-thiazolo(3,2-*a*)pyrimidin-5-one (ritanserin, Sigma, Cat. No. R-103).

Twenty-eight rats (477 ± 7 g BW) received subcutaneous injections of 0.5, 1.5, or 3 mg/kg BW ritanserin (Grignaschi et al., 1993; Neill and Cooper, 1989) dissolved in 0.33 N acetic acid (Massi and Marini, 1987).

2.5. Experiment 2, Trial 2: 2×2 factorial arrangement with ritanserin and LPS

In this trial, 28 rats initially weighing 231 ± 4 g were tested using a 2 × 2 factorial arrangement of LPS and ritanserin (0.5 mg/kg BW) as described above. Five days

later, the trial was repeated with rats switched within main effects (treatment to vehicle/vehicle to treatment) so that no rat received LPS or ritanserin treatment twice.

2.6. Experiment 3: dose–response curve to ketanserin in LPS-treated rats

To determine whether ritanserin attenuated LPS-induced anorexia via blockade of the 5-HT_{2A} or 5-HT_{2C} receptor, we tested the ability of ketanserin, a 5-HT_{2A}-specific receptor antagonist, to attenuate LPS-induced anorexia. Twenty-eight LPS-treated rats (209 ± 7 g BW) were injected subcutaneously with 0, 0.02, 0.2, or 2 mg/kg BW 3-(2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl-2,4(1*H*,3*H*)-quinazolinedione (ketanserin, Sigma, Cat. No. S-006) (Grignaschi et al., 1993) dissolved in 0.9% saline (Hewson et al., 1988; Sugimoto et al., 1996).

2.7. Experiment 4: dose–response curve of RS-102221 in LPS-treated rats

Because ritanserin (5-$HT_{2A/C}$ receptor antagonist), but not ketanserin (5-HT_{2A} receptor antagonist), attenuated LPS-induced anorexia, we tested the role of the 5-HT_{2C} receptor in LPS-induced hypophagia using the 5-HT_{2C} receptor antagonist, a benzenesulfonamide of 8-[5-(5-amino-2,4-dimethoxyphenyl) 5-oxopentyl]-1,3,8-triazaspiro[4.5]decane-2,4-dione) (RS-102221, TORICS Cookson, Avonmouth Bristol, UK). Twenty-eight rats initially weighing 270 ± 2 g were used in this experiment. The 5-HT_{2C} receptor antagonist RS-102221 was dissolved in 10% DMSO in distilled water and injected subcutaneously at a dose of either 0.02, 0.2, or 2 mg/kg BW (Bonhaus et al., 1997).

2.8. Experiment 5, Trial 1: dose–response curve to SB 242084 in LPS-treated rats

To further examine the potential role of the 5-HT_{2C} receptor in LPS-induced anorexia, we chose to test 1*H*-indole-1-carboxyamide,6-chloro-2,3-dihydro-5-methyl-*N*-[6-[(2-methyl-3-pyridinyl)oxyl]-3-pyridinyl]-; 6-chloro-5-methyl-1-[(2-[2-methylpyrid-3-yloxy]pyrid-5-yl)-carbamoyl]indoline (SB 242084, Sigma, Cat. No. S-8061), the first reported selective potent and brain penetrant 5-HT_{2C} receptor antagonist (Kennett et al., 1997). Twenty-eight rats (230 ± 4 g BW) were used in this trial. The 5-HT_{2C} receptor antagonist SB 242084 was dissolved in 20% DMSO in distilled water, and injected intraperitoneally at a dose of either 0.03, 0.3, or 3 mg/kg BW SB 242084 (Kennett et al., 1997).

2.9. Experiment 5, Trial 2: 2×2 factorial arrangement with SB 242084 and LPS

In this trial, 36 rats initially weighing 331 ± 5 g were tested in a 2 × 2 factorial design with LPS and SB 242084 (0.3 mg/kg BW) as described, to investigate the ability of SB 242084 to attenuate LPS-induced anorexia.

2.10. Experiment 6: 2×2 factorial design of metoclopramide and LPS

We also addressed the role of the 5-HT_3 receptor in LPS-induced anorexia using the 5-HT_3 receptor antagonist metoclopramide in a 2 × 2 factorial design with LPS. Metoclopramide (Sigma, Cat. No. M-0763) was dissolved in saline and injected intraperitoneally at a dose of 2 mg/kg BW (Homesley et al., 1993). Twenty-eight rats initially weighing 384 ± 8 g were tested.

2.11. Statistical analysis

Results from dose–response trials were analyzed using General Linear Models (GLM) procedures appropriate for a one-way analysis of variance with blocking. When an overall significant ANOVA occurred, treatment means were compared using Duncan's multiple range test or orthoganal contrasts. 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. Experiment 2, Trial 2 (Ritanserin × LPS trial) was conducted twice, with rats being switched within main effects between trials (treatment to vehicle/vehicle to treatment). In this manner, no rat received LPS or ritanserin treatment twice, so each rat's data for both trials were included in the analysis. In Experiment 5, Trial 2, nine rats per treatment were used. Because the dose–response trials to ritanserin and SB 242084 both yielded marginally statistically significant results, the number of rats/treatment was increased in the subsequent Antagonists × LPS factorial arrangement trials. In all experiments, blocks consisted of rats with similar body weights. Results are expressed as mean ± S.E.M.

3. Results

3.1. Experiment 1: dose–response curve to cyanopindolol and SB 224289 in LPS-treated rats

3.1.1. Trial 1: cyanopindolol

Between 4 and 6 h after injection, rats that received only LPS and vehicle ate 1.4 ± 0.3 g, whereas the same rats without LPS injection usually ate >3 g during that time (data not shown). Subcutaneous administration of the 5-HT_{1B} receptor antagonist cyanopindolol at 0.3 to 0.6 mg/kg did not alter food intake in LPS-treated rats [$F(2,25) = 0.06$, $P < .9$, Fig. 1].

3.1.2. Trial 2: SB 224289

Between 4 and 6 h after injection, rats that received only LPS and vehicle ate 0.4 ± 0.1 g. Intraperitoneal injection of

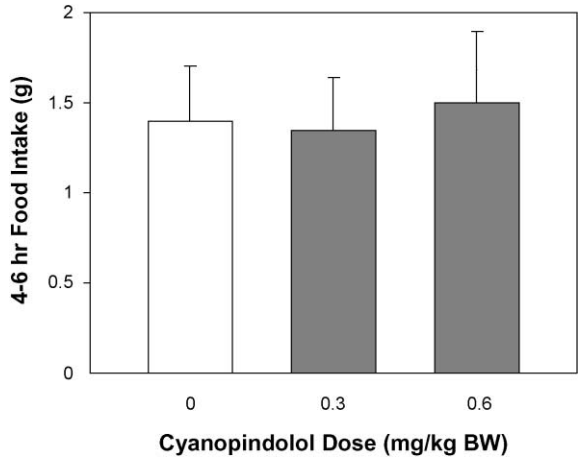

Fig. 1. Food intake of LPS-injected rats in response to cyanopindolol. All rats were injected intraperitoneally with LPS (100 μg/kg BW) just before lights out (0 h). Within 15 min before 4 h, rats received cyanopindolol subcutaneously. Data are mean ± S.E.M. food intakes of nine animals, each, the 2 h following cyanopindolol administration. Groups ate similar amounts of food during the 2 h following cyanopindolol administration regardless of dose ($P < .9$).

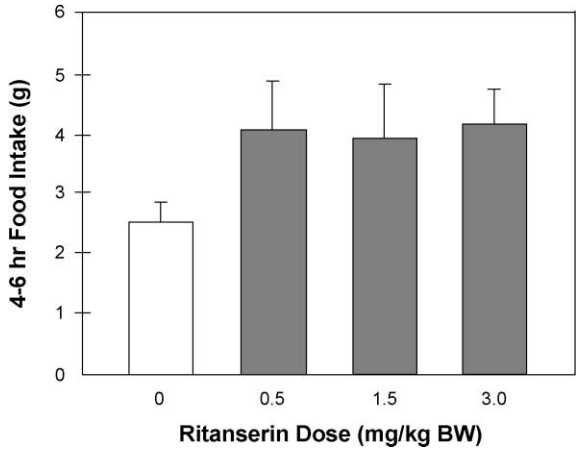

Fig. 3. Food intake of LPS-injected rats in response to ritanserin. All rats were injected intraperitoneally with LPS (100 μg/kg BW) just before lights out (0 h). Within 15 min before 4 h, rats received subcutaneous injections of ritanserin. Data are mean ± S.E.M. food intakes of seven animals, each, for the 2 h following ritanserin administration. Although not statistically significant, all ritanserin groups ate consistently more food than controls ($P < .07$).

the 5-HT_{1B} receptor antagonist SB 224289 at 0.05, 0.5, or 5 mg/kg did not alter food intake in LPS-treated rats [$F(3,23) = 0.17$, $P < .9$, Fig. 2]. The lower food intake of all rats compared to Trial 1 was presumably due to the animals' lower body weight in Trial 2 because the anorectic effect of LPS appears to decrease with increasing body weight (Langhans, 1996).

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

Ritanserin, administered at 0.5, 1.5, and 3 mg/kg BW, appeared to attenuate LPS-induced anorexia similarly regardless of dose. Although all rats that received ritanserin ate an average of 1.7 g more than rats that received only LPS and vehicle during the test period, the difference was only marginally significant [control vs. ritanserin, $F(1,24) = 3.4$, $P < .07$, Fig. 3].

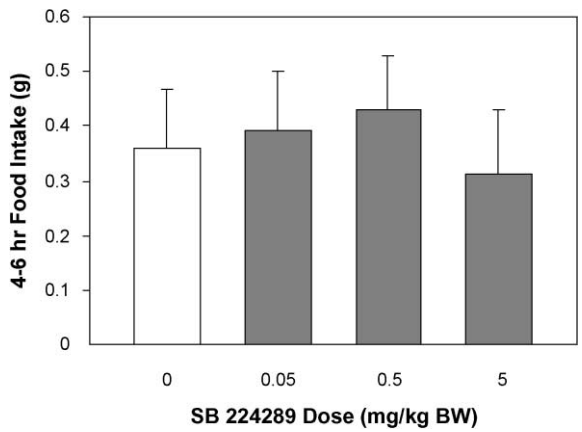

Fig. 2. Food intake of LPS-injected rats in response to SB 224289. All rats were injected intraperitoneally with LPS (100 μg/kg BW) just before lights out (0 h). Within 15 min before 4 h, rats received intraperitoneal injections of SB 224289. Data are mean ± S.E.M. food intakes of seven animals, each, for the 2 h following SB 224289 administration. Groups ate similar amounts of food during the 2 h following SB 224289 administration regardless of dose ($P < .9$).

3.3. Experiment 2, Trial 2: 2×2 factorial arrangement with ritanserin and LPS

LPS caused a significant reduction in food intake during 4–6 h after injection [$F(1,58) = 15.4$, $P < .005$, Fig. 4]. Ritanserin treatment almost completely blocked the LPS-

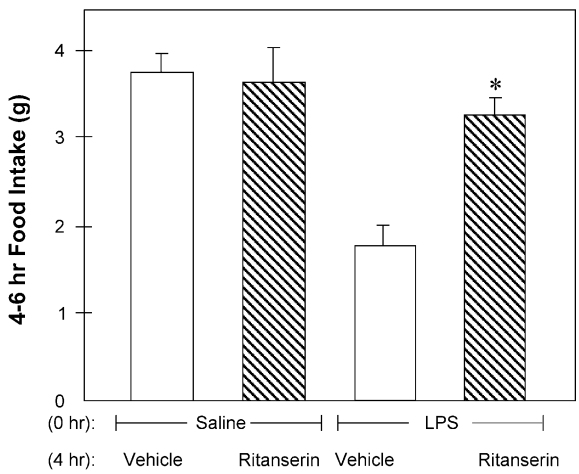

Fig. 4. Ritanserin specifically attenuates LPS-induced anorexia (LPS × Ritanserin interaction: $P < .01$), but does not increase food intake in non-LPS-treated rats. Rats were injected with 100 μg/kg BW LPS ($n = 14$) or saline just before lights out (0 h). At 4 h after LPS, half the rats in each group were injected with ritanserin (0.5 mg/kg) or vehicle. Data are means ± S.E.M. food intakes of 14 rats for the 2 h following ritanserin administration.

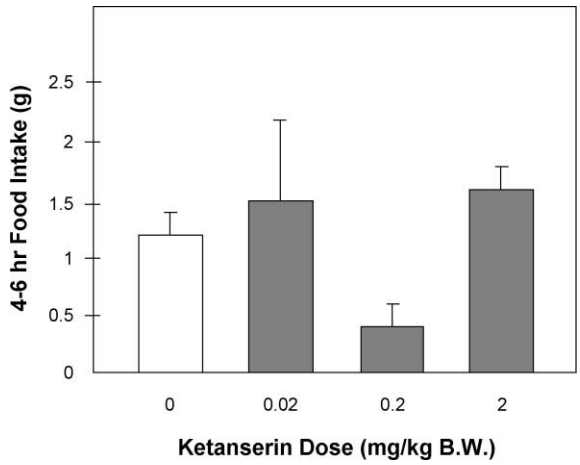

Fig. 5. Food intake of LPS-injected rats in response to ketanserin. All rats were injected intraperitoneally with LPS (100 μg/kg BW) just before lights out (0 h). Within 15 min before 4 h, rats received subcutaneous injections of ketanserin. Data are mean ± S.E.M. food intakes of seven animals, each, for the 2 h following ketanserin administration. All groups ate similar amounts of food during the 2 h following ketanserin administration regardless of dose ($P < .1$).

induced anorexia [$F(1,58) = 6.33$, $P < .01$, Fig. 4], but did not alter food intake in non-LPS-treated rats during the same period.

3.4. Experiment 3: dose–response curve to ketanserin in LPS-treated rats

Between 4 and 6 h after injection, rats that received only LPS and vehicle ate 1.2 ± 0.2 g. Subcutaneous administration of the 5-HT_{2A} receptor antagonist ketanserin at 0, 0.02, 0.2, or 2 mg/kg BW did not alter food intake in LPS-treated rats [$F(3,24) = 2.09$, $P < .1$, Fig. 5].

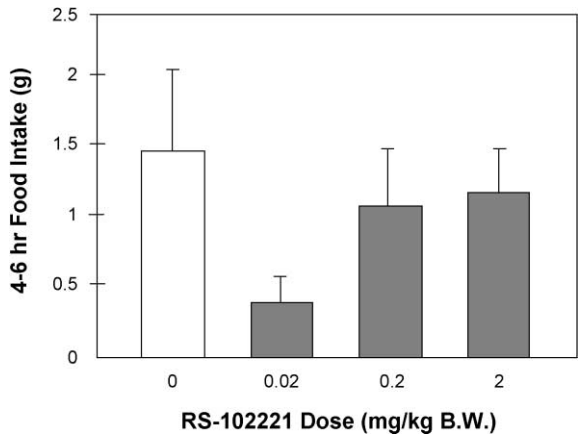

Fig. 6. Food intake of LPS-injected rats in response to RS-102221. All rats were injected intraperitoneally with LPS (100 μg/kg BW) just before lights out (0 h). Within 15 min before 4 h, rats received subcutaneous injections of RS-102221. Data are mean ± S.E.M. food intakes of seven animals, each, for the 2 h following RS-102221 administration. All groups ate similar amounts of food after RS-102221 regardless of dose ($P < .4$).

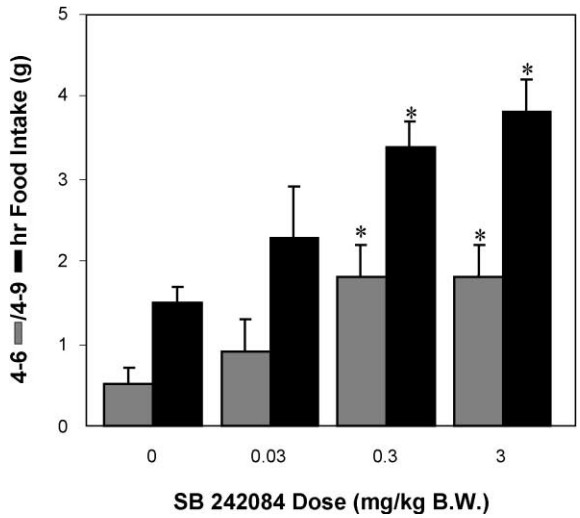

Fig. 7. Food intake of LPS-injected rats in response to SB 242084. All rats were injected intraperitoneally with LPS (100 μg/kg BW) just before lights out (0 h). Within 15 min before 4 h, rats received intraperitoneal injections of SB 242084. Data are mean ± S.E.M. food intakes of nine animals, each, for the 2 h following SB 242084 administration. Rats injected with 0.3 and 3 mg/kg ate significantly more compared to controls from 4–6 h ($P < .05$) as well as 4–9 h ($P < .004$) after LPS.

3.5. Experiment 4: dose–response curve to RS-102221 in LPS-treated rats

Between 4 and 6 h after injection, rats that received only LPS and vehicle ate 1.5 ± 0.6 g. Subcutaneous administration of the 5-HT_{2C} receptor antagonist RS-102221 at 0, 0.02, 0.2, or 2 mg/kg did not alter food intake in LPS-treated rats [$F(3,30) = 1.1$, $P < .4$, Fig. 6].

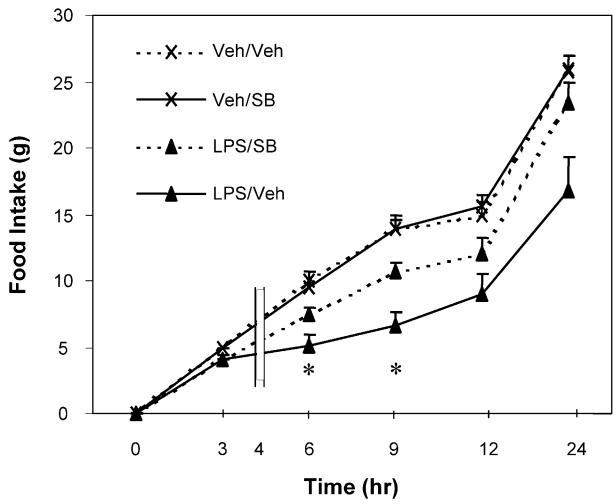

Fig. 8. SB 242084 specifically attenuates LPS-induced anorexia during 4–6 and 4–9 h after administration (* LPS × SB 242084 interaction, both $P < .005$), but does not increase food intake in non-LPS-treated rats. Rats were injected with 100 μg/kg BW LPS ($n = 9$) or saline just before lights out (0 h). At 4 h after LPS, half the rats in each group were injected with SB 242084 (0.3 mg/kg) or vehicle. Data are mean ± S.E.M. 24 h cumulative food intakes of nine animals, each.

3.6. Experiment 5, Trial 1: dose–response curve to SB 242084 in LPS-treated rats

Between 4 and 6 h after injection, rats that received only LPS and vehicle ate 0.6 ± 0.1 g. Intraperitoneal administration of the 5-HT_{2C} receptor antagonist SB 242084 at 0, 0.03, 0.3, or 3 mg/kg resulted in a marginally significant increase in food intake in LPS-treated rats [$F(3,23) = 2.9$, $P < .06$, Fig. 7]. Unlike 8-OH-DPAT, a 5-HT_{1A} receptor agonist, which only attenuated LPS-induced anorexia for 2 h post-injection, SB 242084 continued to enhance feeding up to 5 h after administration (4–9 h after LPS) in LPS-treated rats [$F(3,22) = 2.9$, $P < .06$, Fig. 7] especially at doses of 0.3 and 3 mg/kg (food intake was significantly greater than controls when compared directly). By 24 h after LPS administration, food intake in SB 242084 treated rats was still greater than in controls (4–6 g), but not significantly.

3.7. Experiment 5, Trial 2: 2×2 factorial arrangement with SB 242084 and LPS

LPS caused a significant reduction in food intake during 4–6 h after injection [$F(1,32) = 29.43$, $P < .001$, Fig. 8]. SB 242084 treatment attenuated the LPS-induced anorexia [$F(1,32) = 9.66$, $P < .004$, Fig. 8], but did not alter food intake in non-LPS-treated rats during the same period. As in the dose–response trial, SB 242084 continued to attenuate LPS-induced anorexia in rats from 4 to 9 h [$F(1,31) = 10.85$, $P < .003$, Fig. 8].

3.8. Experiment 6: 2×2 factorial arrangement with metoclopramide and LPS

LPS caused a significant reduction in food intake during 4–6 h after injection [$F(1,24) = 25.94$, $P < .0001$, Fig. 9].

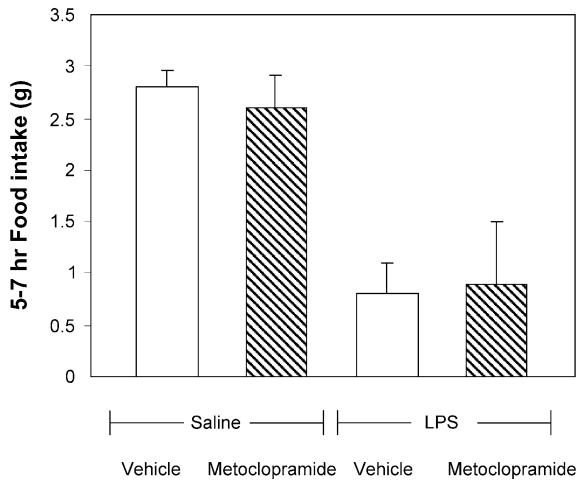

Fig. 9. Metoclopramide does not attenuate LPS-induced anorexia ($P < .6$). Rats were injected with 100 μg/kg BW LPS ($n = 7$) or saline just before lights out (0 h). At 4 h after LPS, half the rats in each group were injected with metoclopramide (2 mg/kg) or vehicle. Data are means ± S.E.M. food intakes of seven rats for the 2 h following metoclopramide administration.

Metoclopramide did not attenuate the LPS-induced anorexia [$F(1,24) = 0.25$, $P < .6$, Fig. 9]. Also, it appeared to decrease food intake in non-LPS-treated rats, but this difference did not reach statistical significance.

4. Discussion

The present experiments were conducted to determine which 5-HT receptor subtype(s), may mediate the hypophagic response following intraperitoneal administration of LPS. In a previous study, we showed that the 5-HT_{1A} receptor agonist 8-OH-DPAT (= functional antagonist) attenuated intraperitoneal LPS-induced hypophagia (Hrupka and Langhans, 2001). Here, we report that 5-HT antagonism via the selective 5-HT_{2C} receptor antagonist SB 242084 and the less specific 5-$HT_{2A/C}$ receptor antagonist ritanserin both attenuated LPS-induced anorexia in rats. These effects were observed during the dark phase, when the same doses of antagonists did not alter food intake in non-LPS-injected control rats. Our results indicate that the 5-HT_{2C} receptor is involved in mediating LPS's feeding effects under pathophysiological conditions. This is consistent with other reports suggesting that the 5-HT_{2C} receptor plays a significant role in food intake regulation under nonpathophysiological conditions (Kennett et al., 1997; Simansky, 1996; Tecott et al., 1995).

Doses of LPS or IL-1β higher than necessary to decrease feeding enhance CNS 5-HT levels and 5-HT turnover (Dunn, 1992) in most brain areas. These changes can be widespread throughout the brain at "high" doses (Dunn, 1992). At low doses, however, LPS and cytokines can cause discrete changes in 5-HT metabolism in specific hypothalamic areas known to be involved in the control of feeding (MohanKumar et al., 1999; Yang et al., 1999). The APR seems to be a condition in which an increase in the tone of the 5-HT system is important in controlling food intake. Because of the increase in CNS 5-HT activity during the APR, LPS-induced anorexia may mimic some of the effects of anorexia induced by the 5-HT releaser and uptake inhibitor D-fenfluramine. For example, pharmacological antagonism or gene knockout of the 5-HT_{1B} (Grignaschi et al., 1993; Lucas et al., 1998; Neill and Cooper, 1989) or 5-HT_{2C} (Clifton, 2000; Neill and Cooper, 1989; Tecott et al., 1995; Vickers et al., 2001) receptor is often, but not always (Lawton and Blundell, 1993; Vickers et al., 2001), reported to attenuate or block D-fenfluramine-induced anorexia. Because several different experimental feeding paradigms (food restriction vs. palatable diet to induce eating) were used to evaluate D-fenfluramine-induced anorexia, it is difficult to generalize these findings to ad libitum chow feeding in rats, the condition under which we examined LPS-induced anorexia. The two conditions are probably different because, in contrast to the feeding suppressive effect of fenfluramine, only 5-HT_{2C} antagonism and not 5-HT_{1B} antagonism attenuated LPS-induced anorexia.

While we observed that ritanserin and SB 242084 consistently attenuated LPS-induced anorexia in rats, Swiergiel and Dunn (2000) reported that ritanserin did not attenuate IL-1β-induced anorexia during the light phase in mice fed a palatable diet. Peripheral LPS administration is known to stimulate immune cells to release cytokines such as IL-1β (Dinarello, 1988), which may then increase serotoninergic (5-HT) activity (Dunn, 1992; MohanKumar et al., 1999) and exert an inhibitory influence upon feeding. In an unpublished experiment, we injected mice with 150 ng IL-1β at lights out and ritanserin (2 mg/kg) immediately thereafter. Consistent with the report by Swiergiel and Dunn (2000), we also observed that ritanserin did not attenuate IL-1β-induced anorexia in chow-fed mice during the dark phase (data not shown). In contrast, we observed that ritanserin attenuated intracerebroventricular IL-1β-induced anorexia in chow-fed rats during the dark phase (von Meyenburg, unpublished data). Thus, we believe the inability of 5-HT_{2C} antagonism to attenuate IL-1β-induced anorexia in mice is likely due to a species difference rather than to a difference between the actions of LPS and IL-1β or in the experimental paradigms (dark vs. light phase, chow vs. sweetened milk). This possibility deserves to be critically examined.

In our hands, the selective 5-HT_{2C} receptor antagonist SB 242084 attenuated LPS-induced anorexia, whereas RS-102221 did not. These results are consistent with reports showing that SB 242084, but not RS-102221, attenuates mCCP-induced (5-$HT_{1B/2C}$ receptor antagonist) hypolocomotion (Bonhaus et al., 1997; Kennett et al., 1997). Repetitive daily injections of RS-102221 (2 mg/kg BW) at light onset have also been reported to enhance food intake in rats (Bonhaus et al., 1997), but we did not observe a significant increase in food intake in control animals with a single injection of RS-102221 at the same dose (2 mg/kg BW) (data not shown). Further studies are necessary to determine whether this difference is related to the different experimental design and/or perhaps to a delayed uptake of RS-102221 across the blood–brain barrier (Bonhaus et al., 1998).

We previously observed that 8-OH-DPAT consistently attenuated LPS-induced anorexia during the 2-h postinjection (Hrupka and Langhans, 2001). Yet, this initial attenuation by 8-OH-DPAT was compensated for by a more pronounced hypophagia between 2 and 4 h after injection, so that overall cumulative food intake after LPS was not altered by 8-OH-DPAT treatment. In the present studies, SB 242084 significantly attenuated LPS-induced anorexia for at least 5 h after administration (4–9 h after LPS, Fig. 8). SB 242084-treated LPS-injected rats ate, in fact, 4–6 g more than rats that received only LPS and vehicle injection by 24 h after injection. Thus, not only does 5-HT_{2C} antagonism markedly and acutely attenuate LPS-induced anorexia, the effect appears to be lasting and does not dissipate within 24 h.

Previous studies suggest the involvement of the 5-HT_{1B} receptor in food intake control (Grignaschi et al., 1995; Kennett and Curzon, 1988; Kennett et al., 1987). In our studies, the 5-$HT_{1A/1B}$ (Grignaschi et al., 1995) receptor antagonist cyanopindolol did not attenuate LPS-induced anorexia at 0.3 or 0.6 mg/kg. Although 0.3 mg/kg has been reported to attenuate D-fenfluramine-induced anorexia (Neill and Cooper, 1989), often higher doses of cyanopindolol (3 or 8 mg/kg) were required to attenuate the food intake reduction induced by D-fenfluramine (Grignaschi et al., 1995) or 5-HT_{1B} agonists (mCCP, TFMPP and Ru 24969 (Kennett et al., 1987). The more selective 5-HT_{1B} receptor antagonist, SB 224289, also failed to attenuate LPS-induced anorexia at doses of 0.05–5 mg/kg BW, although these doses have previously been shown to block 5-HT_{1B} autoreceptor effects (Vickers et al., 2001). Thus, 5-HT_{1B} receptor appears to be not necessary for LPS-induced anorexia.

Although the 5-HT_{2A} and 5-HT_3 receptors appear to be involved in the control of food intake under certain conditions (5-HT_{2A}—Grignaschi et al., 1996; Halford and Blundell, 2000; 5-HT_3—Hammer et al., 1990; Hrupka et al., 1991), we found no effect of either 5-HT_{2A} receptor antagonism via ketanserin or 5-HT_3 receptor antagonism by metoclopramide on LPS-induced anorexia. This suggests that these receptors are also not necessary for LPS-induced anorexia.

In our hands, antagonism of 5-HT_{2C} receptors consistently attenuated LPS-induced anorexia by about 50%, suggesting that LPS-induced anorexia is mediated by other neurotransmitters or neuropeptides as well. In addition to increasing brain 5-HT levels, LPS has been reported to increase the concentration of dopamine (MohanKumar et al., 1999), GLP-1 (Rinaman, 1999), αMSH (Catania et al., 1995) and peripheral hormones such as leptin (Faggioni et al., 1997), which are all implicated in the regulation of food intake in rats (Faggioni et al., 1997; Huang et al., 1999; Rinaman and Comer, 2000). As all these neurochemicals may also be involved in LPS-induced anorexia, a simultaneous blockade of several receptors may be necessary to completely prevent LPS-induced anorexia (Swiergiel and Dunn, 2000).

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