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Evidence for a role of the 5-HT_{2C} receptor in central lipopolysaccharide-, interleukin-1β-, and leptin-induced anorexia

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Abstract

We examined the role of serotonin (5-HT) and the 5-HT_{1A} and 5-HT_{2C} receptors in the anorectic effects of centrally administered lipopolysaccharide (LPS), interleukin-1β (IL-1β), and leptin. Food intake was measured in rats after intracerebroventricular (ICV) injections of LPS (20 ng), IL-1β (10 ng), or leptin (1μg) at lights out, followed by intraperitoneal (IP) injections of either the 5-HT_{1A} autoreceptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetraline (8-OH-DPAT) (125 μg/kg) or the 5-HT_{2C} receptor antagonist SB 242084 (0.3 mg/kg) at the onset of anorexia. SB 242084 significantly attenuated the food intake reduction caused by all compounds (all $P<.01$). IP 8-OH-DPAT attenuated ICV IL-1β-induced anorexia ($P<.01$). We also tested the involvement of the median raphe 5-HT_{1A} receptors in peripheral LPS- and IL-1β-induced anorexia. Rats were injected intraperitoneally with either LPS (100 μg/kg) or IL-1β (2 μg/kg) at lights out, and 8-OH-DPAT (4 nmol) was administered directly into the median raphe nucleus at the onset of anorexia. Median raphe injections of 8-OH-DPAT significantly attenuated both IL-1β- and LPS-induced anorexia (both $P<.01$). These results implicate the 5-HT_{2C} receptors in the mediation of central LPS-, IL-1β-, and leptin-induced anorexia. Our results also suggest that the midbrain raphe nuclei play a role in mediating the anorectic response to peripheral LPS and IL-1β.

Keywords: Serotonin; 8-OH-DPAT; SB242084; Raphe nucleus

1. Introduction

Microbial infections often result in multiple physiological and behavioral changes, including lethargy and anorexia. These changes are part of the host's acute phase response (APR) and are also observed after administration of bacterial lipopolysaccharide (LPS). LPS is located in the outer membrane of most gram-negative bacteria (Hart, 1988), and its administration is used as an animal model of acute gram-negative bacterial infection or inflammation (Tilders et al., 1994). Natural infections and LPS administration both stimulate the release of proinflammatory cytokines, such as interleukin-1β (IL-1β), from immune cells (Dinarello, 1988). IL-1β also reduces food intake after parenteral administration and may therefore be one mediator of LPS's anorectic effect. Both LPS and IL-1β increase serotonergic (5-HT) activity in the brain when injected peripherally (Dunn, 1992; Dunn et al., 1999) or centrally (Plata-Salaman and Borkoski, 1993; Zubareva et al., 2001). Increased 5-HTergic activity in the brain has been shown to inhibit feeding (Blundell, 1986; Currie and Coscina, 1996; Curzon, 1990; Simansky, 1996), and numerous pharmacological and behavioral studies indicate that drugs which act on the 5-HTergic system do influence food intake (Cerulli et al., 1998; Garattini, 1995). We previously reported that the specific 5-HT_{1A} autoreceptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetraline (8-OH-DPAT), and the specific 5-HT_{2C} receptor antagonist 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) both attenuate the anorexia following peripheral administration of LPS in rats (Hrupka and Langhans, 2001; von Meyenburg et al., 2003), suggesting that an increase in 5-HTergic activity and 5HT_{2C} receptor activation are involved in mediating the feeding suppressive effect of peripheral LPS. LPS and cytokines can alter meal patterns differently when injected

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peripherally or centrally, suggesting they may be acting on different CNS feeding mechanisms depending on the route of administration (Langhans, 1996). Further, the feeding suppressive effects of IL-1β and LPS may in part be mediated by different neurochemical systems (Langhans et al., 1993). We therefore investigated whether 8-OH-DPAT and SB 242084 would also attenuate the anorexia induced by intracerebroventricular (ICV) administration of LPS, IL-1β, or leptin.

Leptin was included in this study because (1) LPS and cytokines stimulate leptin release from adipocytes (Grunfeld et al., 1996; Sarraf et al., 1997); (2) leptin also increases 5-HTergic activity in the brain (Calapai et al., 1999); and (3) the leptin receptors share functional similarities with the interleukin-6 receptor family (Fantuzzi and Faggioni, 2000).

The midbrain raphe is a major site of origin of 5-HTergic neurons projecting to the forebrain. Injection of 8-OH-DPAT into the midbrain raphe nuclei in rats increases food intake (Currie and Coscina, 1993). Antagonism of the 5-HT_{1A} somatodendritic autoreceptors enhances feeding by suppressing 5-HT cell firing and inhibiting 5-HT synthesis and release (Currie et al., 1998; Hjorth and Magnusson, 1988; Sprouse and Aghajanian, 1987). To determine whether 5-HTergic neurons in the raphe nucleus are involved in mediating the anorexia during infections, we also tested whether 8-OH-DPAT administered into the medial raphe nucleus blocked the anorexia in response to intraperitoneal (IP) LPS and IL-1β injections.

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 specific 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 Canton of Zurich's Animal Use and Care Committee.

2.2. Implantation of brain cannulae/infusions

Rats were allowed to acclimate to the vivarium for at least 1 week prior to surgery, then, they were food-deprived over night and anesthetized with 80 mg/kg ketamine HCl (Narketan 10, Chassot, Bern, Switzerland) plus 4 mg/kg xylazine (Rompun, Bayer, Leverkusen, Germany) for implantation of lateral ventricle and median raphe guide cannulae. A 24-gauge stainless-steel guide cannula was implanted 1 mm above the lateral ventricle at these coordinates: AP 0.8 mm, L 1.5 mm, and V 1.5 mm from dura according to the atlas of Palkovits and Brownstein (1988), or 4 mm above the median raphe nucleus. The median raphe nucleus guide cannulae were implanted at an angle of 20° to the vertical according to the following coordinates relative to interaural zero: AP +1.2 mm, L −1.2 mm, and V +5.5 mm. Guide cannulae were secured with acrylic cement and three stainless-steel screws that penetrated the scull. All cannulae were fitted with stainless-steel inner stylets to maintain patency. Rats were allowed at least 10 days to recover before experimental testing.

Intracerebroventricular/Raphe nucleus microinfusions were made via a 31-gauge stainless-steel injection needle that was connected through a tygon tube to a 10-μl Hamilton microsyringe. Infusions into the lateral ventricle (5 μl volume) or raphe nucleus (0.4 μl volume) were done over a period of about 2 min. Injection cannulae were left in place for a short while after infusion to prevent any injectate reflux. A period of 3–4 injection-free days was allowed between successive tests.

2.3. Drugs

Drugs used included LPS from *Escherichia coli,* serotype 0111:B4 (No L-2630, Sigma) diluted in 0.9% saline, recombinant human IL-1β (R&D Systems, UK) diluted in PBS/BSA, recombinant human leptin (No. L-4146, Sigma) diluted in 0.9% saline, the 5-HT_{1A}-autoreceptor agonist 8-OH-DPAT (Sigma, No L-57H4131) diluted in 0.9% saline or artificial CSF (aCSF) when injected into the raphe nucleus, and the specific 5-HT_{2C}-receptor antagonist SB 242084 (Sigma, No L-060K4608) diluted in 20% DMSO (v/v) in distilled water. Intraperitoneally injected compounds were delivered in a volume of 0.1 ml/kg body weight. Similar doses as in previous experiments were used (Hrupka and Langhans, 2001; von Meyenburg et al., 2003).

2.4. Experimental design: 2×2 factorial arrangements of ICV LPS, IL-1β or leptin vs. IP 8-OH-DPAT or SB 242084 (Experiments 1–3)

A separate group of rats was used for the 8-OH-DPAT and SB 242084 trials. The same group of rats was used, however, to test each drug's ability to restore feeding following ICV LPS, IL-1β, and leptin administration. Thus, each rat received ICV LPS, IL-1β, and leptin (in that order) in a series of three trials. Our previous studies show that prior LPS administration does not affect the anorectic effect of IL-1β (Langhans et al., 1993; Porter et al., 1998). Preliminary unpublished studies indicate that this also holds for leptin. For each trial, rats were divided into 4 groups according to body weight, and a 2 × 2 factorial arrangement of LPS, IL-1β, or leptin vs. 5-HT drug injection was used. To increase the number of animals/treatment, rats were switched within the main effects (LPS/IL-1β/leptin↔vehicle; 5-HT

drug↔vehicle), and 3–4 days later, the trial was conducted a second time. In this manner, no rat received the same combination of 'anorectic agent' (LPS, IL-1β, leptin) and 5-HT drug twice. This was done in all experiments except for Trials 1b, 4a, and 4b. Food intake was measured by weighing the feeding cups (±0.1 g) and correcting for spillage. Body weight after treatment was not recorded systematically because previous studies suggested that it remains unchanged in short-term experiments with such doses of LPS, IL-1β, and leptin.

2.4.1. Experiment 1 (a/b) ICV LPS+8-OH-DPAT/SB 242084

Fourteen (369±5 g body weight, mean±S.E.M.) rats/treatment were used to test the effect of 8-OH-DPAT (functional antagonist) (Trial 1a) on ICV LPS-induced anorexia, while eight (289±4 g body weight, mean±S.E.M.) rats/treatment were used to test the effect of SB 242084 (Trial 1b). Rats received an intracerebroventricular injection of LPS (20 ng/rat diluted in 5 μl of saline) or saline at lights out. Four hours after intracerebroventricular injections, rats were injected with either 8-OH-DPAT (125 μg/kg), SB 242084 (0.3 mg/kg), or the appropriate vehicle. Food intake was recorded at 4, 6, and 24 h. In Trial 1b food intake was also recorded at 8 and 12 h.

2.4.2. Experiment 2 (a/b): ICV IL-1β+8-OH-DPAT/SB 242084

Fifteen (390±2 g body weight, mean±S.E.M.) rats/treatment were used to test the effect of 8-OH-DPAT (Trial 2a) on ICV IL-1β-induced anorexia, while 14 (329±2 g body weight, mean±S.E.M.) rats/treatment were used to test the effect of SB 242084 (Trial 2b). Rats received an intracerebroventricular injection of IL-1β (10 ng/rat diluted in 5 μl PBS/BSA) (Plata-Salaman et al., 1996) or PBS/BSA vehicle at lights out. IL-1β administration causes a reduction in food intake already 2 h after injection (Blundell, 1984; Blundell, 1986; Langhans et al., 1993), so immediately after intracerebroventricular injections, rats were injected intraperitoneally with either 8-OH-DPAT (125 μg/kg), SB 242084 (0.3 mg/kg), or the appropriate vehicle. Food intake was always recorded at 2 and 4 h.

2.4.3. Experiment 3 (a/b): ICV Leptin+8-OH-DPAT/SB 242084

Thirteen (434±8 g body weight, mean±S.E.M.) rats/treatment were used to test the effect of 8-OH-DPAT (Trial 3a) on ICV leptin-induced anorexia, while 12 (340±4 g body weight, mean±S.E.M.) rats/treatment were used to test the effect of SB 242084 (Trial 3b). Rats received an intracerebroventricular injection of leptin (1 μg/rat diluted in 5 μl of saline) (Calapai et al., 1999; Flynn et al., 1998) or saline at lights out. At 3 h after intracerebroventricular injections rats were injected peripherally with either 8-OH-DPAT (125 μg/kg), SB 242084 (0.3 mg/kg), or the appropriate vehicle. Food intake was always recorded at 3 and 5 h.

2.4.4. Experiment 4 (a/b): IP LPS or IL-1β+raphe nucleus injections of 8-OH-DPAT

Eight (322±4 g body weight, mean±S.E.M.) rats/treatment were injected intraperitoneally with either LPS (100 μg/kg body weight) and six rats/treatment were injected with IL-1β (2 μg/kg body weight) or vehicle just before lights out (h 0). 8-OH-DPAT (0.4 nmol/rat diluted 0.4 μl in aCSF) (Currie and Coscina, 1993) or aCSF was administered directly into the raphe nucleus between 3 and 4 h after LPS injection or immediately after IL-1β injection, and food intake was recorded at 4, 6, and 24 h (LPS trial), or at 2, 4, and 24 h (IL-1β trial), respectively. At the end of the experiment, rats were deeply anesthetized with an overdose of sodium pentobarbital and a volume of 0.4 μl of Fast green dye (ink) was infused into the injection site. Animals were then perfused transcardially with a solution of $NaNO_2$ (0.5%), saline (0.9%), and heparin (10 IE/ml, Liquemin, B1020, 25000 IE/UI 5 ml, Roche Pharma, Reinach, Switzerland) followed by formaldehyde, and brains were extracted. After fixation, brains were frozen, cut in a cryostat at 40 μm sections, and subsequently stained with Cresyl violet for histological verification of cannula placement.

2.5. Statistical analysis

Results from LPS/IL-1β/Leptin × 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 experiments in which rats were switched within main effects and injected a second time, each rat's data for both trials and day of trial were included in the analysis. In all experiments, blocks consisted of rats with similar body weights. The ability of 8-OH-DPAT or SB 242084 to attenuate LPS-, IL-1β-, or leptin-induced anorexia was noted by a significant ($P<.05$) interaction between main effects. Results are expressed as means±S.E.M.

3. Results

3.1. Experiment 1 (a/b): ICV LPS+8-OH-DPAT/SB 242084

During the 0–4-h period after administration of LPS or saline, average food intake of both groups was between 4.5 and 5.5 g regardless of treatment. ICV LPS reduced food intake significantly in both trials (all $P<.02$) (Fig. 1). 8-OH-DPAT appeared to attenuate the ICV LPS-induced anorexia between 4 and 6 h (0–2 h after 8-OH-DPAT), but this difference was not significant [$F(1,50)=3.01$, $P<.09$] (Fig. 1a).

SB 242084 administration significantly attenuated LPS-induced anorexia during the first 4 h after its administration, i.e., between 4 and 8 h after LPS [$F(1,26)=7.67$, $P<.01$] (Fig. 1b). Four to twenty-four hours cumulative food intake was still significantly greater in LPS/SB 242084-treated rats than in LPS/vehicle-treated rats [$F(1,26)=4.95$, $P<.04$] (data not shown).

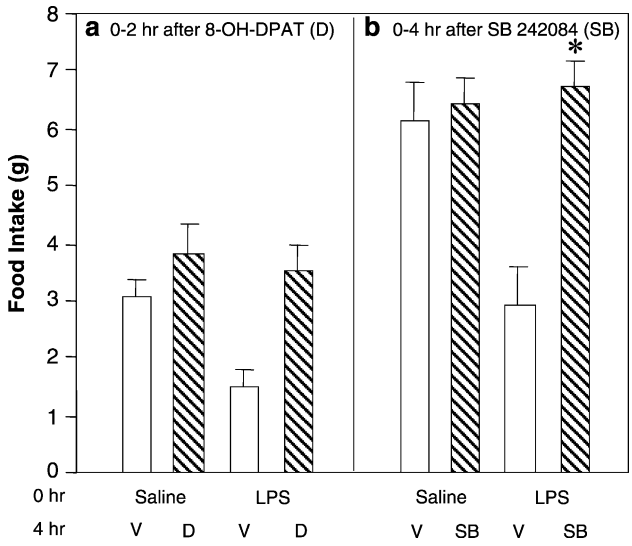

Fig. 1. Effect of (a) 8-OH-DPAT and (b) SB 242084 on the anorexia induced by ICV LPS ($P<.03$). Rats received LPS injections at lights out and 8-OH-DPAT or SB 242084 injections 4 h later. Values are mean ± S.E.M. food intakes of (a) 14 or (b) 8 animals/treatment for the 2 h following 8-OH-DPAT and for the 4 h following SB 242084 administration. * Significant attenuation of LPS-induced anorexia ($P<.01$). V = vehicle, D = 8-OH-DPAT, SB = SB242084.

3.2. Experiment 2 (a/b): ICV IL-1β+8-OH-DPAT/SB 242084

ICV IL-1β reduced food intake significantly in all trials ($P<.004$) (Fig. 2). This effect was already present at 2 h after injection. 8-OH-DPAT attenuated ICV IL-1β-induced anorexia [$F(1,56)=7.06$, $P<.01$] (Fig. 2a) only within the first 2 h. Thereafter, 8-OH-DPAT did not attenuate the IL-1β-induced suppression of feeding (2–4 h food intake in PBS/saline: 2.6 g, PBS/8-OH-DPAT: 2.1 g, IL-1β/saline: 0.5 g, IL-1β/8-OH-DPAT: 0.3 g). 8-OH-DPAT did not affect food intake in non-IL-1β-treated rats.

SB 242084 attenuated 0–2 h IL-1β-induced anorexia [$F(1,49)=7.55$, $P<.008$] (Fig. 2b), but did not affect food intake during the same period in vehicle-treated rats. The effect of SB 242084 disappeared thereafter (2–4 h food intake in PBS/saline: 3 g, PBS/SB 242084: 3.8 g, IL-1β/saline: 0.9 g, IL-1β/SB 242084: 0.6 g).

3.3. Experiment 3(a/b/c): ICV leptin+DPAT/SB 242084

During the 0–3-h period after leptin or saline administration, average food intake of both groups was between 4.0 and 4.5 g regardless of treatment. ICV leptin reduced food intake in all trials between 3 and 5 h ($P<.03$) (Fig. 3). 8-OH-DPAT appeared to attenuate the ICV leptin-induced anorexia between 3 and 5 h, but this difference was not significant [$F(1,50)=0.51$, $P<.5$] (Fig. 3a).

SB 242084 attenuated leptin-induced anorexia significantly between 3 and 5 h [$F(1,46)=6.43$, $P<.01$] (Fig. 3b) and did not alter food intake during the same period in non-leptin-treated rats.

3.4. Experiment 4(a/b): IP LPS or IL-1β+8-OH-DPAT into the median raphe nucleus

Histological verification of cannulae placement revealed that all raphe nucleus cannulae were in the correct place.

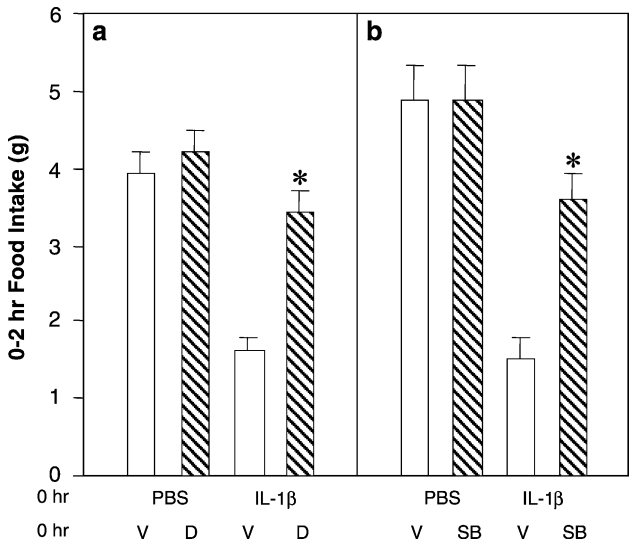

Fig. 2. Effect of (a) 8-OH-DPAT and (b) SB 242084 on the anorexia induced by ICV IL-1β ($P<.0001$). Rats received IL-1β injections at lights out and 8-OH-DPAT or SB 242084 injections immediately thereafter. Values are mean ± S.E.M. food intakes of (a) 15 or (b) 14 animals/treatment for the 2 h following 8-OH-DPAT or SB 242084 administration. * Significant attenuation of IL-1β-induced anorexia ($P<.01$). V = vehicle, D = 8-OH-DPAT, SB = SB242084.

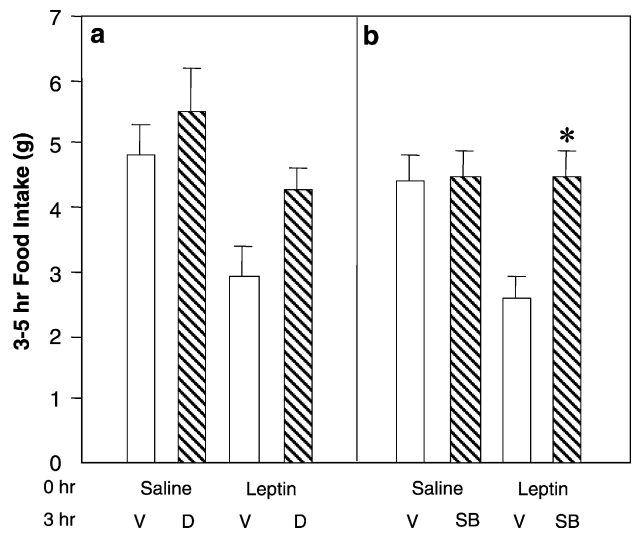

Fig. 3. Effect of (a) 8-OH-DPAT and (b) SB 242084 on the anorexia induced by ICV leptin ($P<.03$). Rats received leptin injections at lights out and 8-OH-DPAT or SB 242084 injections 3 h later. Values are mean ± S.E.M. food intakes of (a) 14 or (b) 12 animals/treatment, for the 2 h following 8-OH-DPAT or SB 242084 administration. * Significant attenuation of leptin-induced anorexia ($P<.01$). V = vehicle, D = 8-OH-DPAT, SB = SB242084.

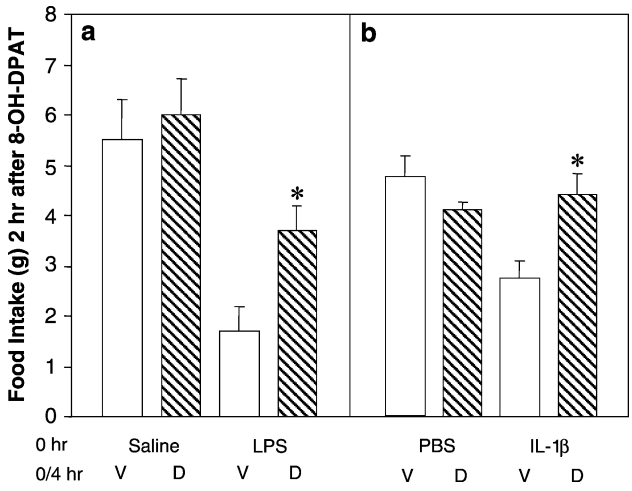

Fig. 4. Effect of 8-OH-DPAT injected into the median raphe nucleus on the anorexia induced by either (a) IP LPS ($P<.0002$) or (b) IP IL-1β ($P<.04$). Rats received LPS or IL-1β injections at lights out and 8-OH-DPAT injections either 4 h later (a) or immediately thereafter (b). Values are means ± S.E.M. food intakes of 8 (a) or 6 (b) animals/treatment, for the 2 h following 8-OH-DPAT administration. * Significant attenuation of LPS and IL-1β-induced anorexia ($P<.01$). V = vehicle, D = 8-OH-DPAT.

During the 0–4-h period after LPS and saline treatment, average food intake was 4.0 g regardless of treatment. LPS reduced 4–6 h food intake compared to control rats [$F(1,29)=18.40$, $P<.0002$], and IL-1β reduced 0–2 h food intake compared to control rats [$F(1,21)=4.96$, $P<.04$]. 8-OH-DPAT injected into the raphe nucleus attenuated the anorexia induced by both LPS and IL-1β (LPS: [$F(1,15)=7.75$, $P<.01$], IL-1β: [$F(1,11)=14.81$, $P<.003$]) (Fig. 4).

4. Discussion

The present experiments were conducted to examine whether the 5-HT_{2C} receptor is important in the anorexia induced by central administration of LPS, IL-1β, and leptin. Our results indicate that blockade of 5-HT_{2C} receptors via the 5-HT_{2C} receptor antagonist SB 242084 or inhibiting 5-HT transmission via the 5-HT_{1A} autoreceptor agonist 8-OH-DPAT, both attenuated central LPS- and IL-1β-induced anorexia. These results are consistent with, and extend, our previous findings (von Meyenburg et al., 2003) showing that 5-HT_{2C} receptor blockade can attenuate peripheral LPS-induced anorexia.

The fact that inhibiting 5-HT neurotransmission attenuates both IP and ICV LPS-induced anorexia is interesting, given that ICV vs. IP LPS administration induces anorexia by distinctly different changes in meal patterns. Peripheral LPS (dose 100 μg/kg) reduces food intake by decreasing meal frequency (Langhans et al., 1989), while ICV LPS (1 ng) (Plata-Salaman and Borkoski, 1993) and IL-1β (0.5–4 ng) (Plata-Salaman, 1994) decrease food intake by decreasing meal size. This suggests that potentially different mechanisms, anatomical and/or neurochemical, may mediate the two responses. For example, IP vs. ICV LPS administration can cause different patterns of CNS *c-fos* induction (Wan et al., 1993) as well as different 5-HT release (Dunn, 1992). This suggests that while centrally vs. peripherally administered LPS and IL-1β may decrease food intake by activating potentially different mechanisms, both seem to converge on the 5-HTergic system as a final common pathway to inhibit feeding.

An important issue is whether 5-HT receptor blockade in LPS-treated rats specifically antagonizes LPS-mediated anorexia or simply causes a nonspecific enhancement in food intake. 5-HT antagonists usually enhance feeding when food intake is low and 5-HT levels are high (e.g., during the light phase) (Bonhaus et al., 1997). Our results indicate that the enhancement in food intake following LPS administration is specifically due to antagonism of an LPS-triggered 5-HT effect because non-LPS-treated rats given the 5-HT_{2C} receptor antagonist did not increase food intake when compared to control rats. For instance, neuropeptide Y (NPY) administration increased food intake in IL-1β-treated rats but also in control animals, suggesting that the feeding stimulatory effect of NPY is not specifically due to antagonism of an IL-1β-triggered effect (Gayle et al., 1997; Sonti et al., 1996).

Although we administered our 5-HT antagonists intraperitoneally, it is reasonable to assume that they affect food intake in response to LPS, IL-1β, and leptin via CNS mechanisms. First, there is little evidence for the expression of 5-HT_{2C} receptors outside the CNS (Barnes and Sharp, 1999). Second, peripheral 5-HT effects on food intake are thought to be mediated via 5-HT_3 receptors and transmitted to the brain via the vagus (Bucinkskaite et al., 2002; Horn and Friedman, 2002). In our laboratory, however, neither 5-HT_3 blockade nor vagal deafferentation altered peripheral LPS-induced anorexia (von Meyenburg et al., 2003; Porter et al., 1998). Third, we were able to significantly attenuate peripheral LPS-, or IL-1β-induced anorexia via 8-OH-DPAT treatment directly into the median raphe nucleus. This also indicates that it is central rather than peripheral 5-HT that mediates peripheral LPS-induced anorexia. It is unlikely that sufficient quantities of 8-OH-DPAT leaked from the brain into the periphery to attenuate the anorexic effect of peripheral LPS or IL-1β via the 5-HT_{1A} receptors in the gut (Barnes and Sharp, 1999).

CNS 5-HTergic neurons are mainly located in the midbrain raphe nuclei (Jacobs and Azmitia, 1992; Mezey et al., 1984) and project to widespread regions in the CNS, including the hypothalamus (Jacobs and Azmitia, 1992; Saavedra et al., 1974; Sawchenko et al., 1983). The 5-HTergic neurons in the raphe nucleus have been found to be important in the regulation of food intake under nonpathological conditions (Currie and Coscina, 1993). Intraraphe and systemic 8-OH-DPAT administration (Bendotti and Samanin, 1986) increases food intake by activating raphe serotonergic 5-HT_{1A} autoreceptors. Activation of these receptors reduces 5-HT synthesis and release (Gardier et al., 1996) in several brain areas, including hypothalamic sites (Sharp and Hjorth,

1990). Our results suggest that these 5-HTergic neurons, originating in the raphe nucleus, are also involved in peripheral LPS- and-IL-1β-induced anorexia. Interestingly, there seems to be a close interaction between the raphe nucleus and the arcuate nucleus of the hypothalamus (Heisler et al., 2002) where the melanocortin system may be a downstream mediator of the 5-HT-induced anorexia.

Neurons in the raphe nucleus can be affected by inflammatory mediators such as cytokines (IL-1β) and prostaglandins (prostaglandin E_2)(Gemma et al., 1991; Nakamura et al., 2002), and these inflammatory mediators are potential neuromodulators involved in peripheral LPS-induced anorexia (Dinarello, 1988; Johnson et al., 2002). The raphe nucleus contains neuronal cells exhibiting IL-1β-immunoreactivity (Gemma et al., 1991) as well as prostaglandin EP3 receptor-like immunoreactivity (Nakamura et al., 2000). The prostaglandin EP3 receptor subtype is believed to mediate large portions of diverse physiologic actions of prostaglandin E_2 (Ek et al., 2000; Nakamura et al., 2000). It would therefore be interesting to test whether the IL-1β and the prostaglandin E_2 receptors in the raphe nucleus are important in the mediation of LPS-induced anorexia.

Our results also support a role for 5-HT and the 5-HT_{2C} receptor in central leptin-induced anorexia. SB 242084 significantly attenuated leptin-induced anorexia and 8-OH-DPAT administration caused a marginally significant attenuation of leptin's effect. These results are consistent with other studies showing that leptin receptors are present on ascending 5-HTergic neurons (Hay-Schmidt et al., 2001) and that leptin increases 5-HT turnover in the brain (Calapai et al., 1999; Hastings et al., 2002). Further evidence for a link between 5-HT and leptin in control of food intake is derived from findings that both 5-HT and leptin modulate the action of NPY (Dryden et al., 1995; Sahu, 1998) as well as the anorexigenic α-melanocyte-stimulating hormone (Heisler et al., 2002; Mizuno et al., 1998). On the other hand, in an in vitro study done by Orlando et al. (2001), leptin was found to not acutely affect hypothalamic 5-HT release from hypothalamic synaptosomes (Orlando et al., 2001), which seems to argue against a role of the 5-HTergic pathway in the acute hypothalamic effect of leptin (Orlando et al., 2001). Further, 5-HT_{2C} receptor gene mutant mice had a normal hypophagic response to exogenous leptin administration, suggesting that the 5-HT_{2C} receptor is not necessary for leptin-induced food intake suppression in mice (Nonogaki et al., 1998). This discrepancy might be due to developmental compensation, during which the hypothalamic neuropeptide mediators of leptin's effects on food intake gain more importance. Thus, there are discrepant findings which hamper the understanding of the Leptin × 5-HT interaction, but our findings indicate that central 5-HT, especially via the 5-HT_{2C} receptor, is involved in the mediation of leptin-induced anorexia.

In sum, the present results provide evidence for a role of the 5-HT_{2C} receptor in the mediation of the anorexia caused by centrally acting LPS, IL-1β, and leptin in the rat. In our hands, antagonism of 5-HT_{2C} receptors consistently attenuated LPS-, IL-1β-, and leptin-induced anorexia by at least 75%. Further studies are needed to examine the situational variability of these effects as well as the interactions with further potential mediators, such as other neurotransmitters (dopamine, histamine) and neuropeptides (α-MSH, CART, CRF, GLP-1) (for review, see Langhans and Hrupka, 2003).

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