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Evidence that PGE2 in the dorsal and median raphe nuclei is involved in LPS-induced anorexia in rats

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ABSTRACT

Anorexia is an element of the acute-phase immune response. Its mechanisms remain poorly understood. Activation of inducible cyclooxygenase-2 (COX-2) in blood–brain-barrier endothelial cells and subsequent release of prostaglandins (e.g., prostaglandin E2, PGE2) may be involved. Therefore, we sought to relate the effects of prostaglandins on the anorexia following gram-negative bacterial lipopolysaccharide treatment (LPS) to neural activity in the dorsal and median raphe nuclei (DRN and MnR) in rats. COX-2 antagonist (NS-398, 10 mg/kg; IP) administration prior to LPS (100 μg/kg; IP) prevented anorexia and reduced c-Fos expression the DRN, MnR, nucleus tractus solitarii and several related forebrain areas. These data indicate that COX-2-mediated prostaglandin synthesis is necessary for LPS anorexia and much of the initial LPS-induced neural activation. Injection of NS-398 into the DRN and MnR (1 ng/site) attenuated LPS-induced anorexia to nearly the same extent as IP NS-398, suggesting that prostaglandin signaling in these areas is necessary for LPS anorexia. Because the DRN and MnR are sources of major serotonergic projections to the forebrain, these data suggest that serotonergic neurons originating in the midbrain raphe play an important role in acute-phase response anorexia.

1. Introduction

Bacterial infections and other immune challenges are met by a complex immune reaction called the acute-phase response (APR). Anorexia is a prominent element of the APR, and investigation of the anorectic effect of the bacterial toxin lipopolysaccharide (LPS) is widely used to investigate the APR's peripheral and central mechanisms. LPS is a component of the outer lipid layer of gram-negative bacteria and is released into the circulation during bacterial reproduction or lysis. LPS binding to toll-like receptor-4 in macrophages and other immune cells causes the release of pro-inflammatory signaling molecules, such as cytokines and prostaglandins (Takeda and Akira, 2005), which in turn initiate immune–neuro-endocrine cascades that ultimately lead to the APR (reviewed in Asarian and Langhans, 2010).

A signaling cascade that appears crucial to brain-mediated elements of the APR involves induction of cyclo-oxygenases (e.g., COX-2) and prostaglandin H synthase, which catalyze the synthesis of prostaglandin E2 (PGE2) and other prostaglandins (Goppelt-Struebe, 1995). For example, COX-2 mRNA and protein are markedly induced in rat brain perivascular microglia and endothelial cells after LPS administration (Cao et al., 1997; Elmquist et al., 1997; Schiltz and Sawchenko, 2002; Yamagata et al., 2001). Tests of selective inhibitors of COX-1, which is not inducible, and COX-2 indicate that only COX-2 is crucial for LPS anorexia in both rats and mice (Johnson et al., 2002; Lugarini et al., 2002). That is, pretreatment of rats with a COX-1 inhibitor did not attenuate LPS anorexia, whereas pretreatment with the COX-2 inhibitor *N*-[2-(Cyclohexyloxy)-4-nitrophenyl] methanesulfonamide (NS-398) did (Lugarini et al., 2002). Similarly, LPS anorexia was reversed in mice with genetic deletions of COX-2, but not of COX-1 (Swiergiel and Dunn, 2001).

How PGE2 acts in the brain to elicit LPS-anorexia is still unknown. One possibility is that it acts on serotonin neurons. This is suggested by the facts that serotonin antagonists reduce LPS anorexia (Hrupka and Langhans, 2001; von Meyenburg et al., 2003a) and that one subtype (EP3) of prostaglandin receptors is abundantly expressed in midbrain serotonergic neurons (Nakamura et al., 2000). Here we (1) further tested the hypothesis that brain PGE2 mediates the anorexia produced by intraperitoneal (IP) injections of LPS in rats using IP injections of the potent and specific COX-2 antagonist NS-398; (2)

Abbreviations: 8-OH-DPAT, 8-hydroxy-*N*,*N*-dipropyl-2-aminotetralin; A1, A1 area of the ventrolateral medulla; APR, acute-phase response; Arc, arcuate nucleus of the hypothalamus; CeA, central nucleus of the amygdala; COX, cyclooxygenase; CRH, corticotropin-releasing hormone; DRN, dorsal raphe nucleus; EP3, a prostaglandin receptor subtype; ICV, intracerebroventricular; IP, intraperitoneal; LPS, bacterial lipopolysaccharide; MnR, median raphe nucleus; NS-398, *N*-[2-(Cyclohexyloxy)-4-nitrophenyl] methanesulfonamide; NTS, nucleus tractus solitarii; PB, phosphate buffer; PGE2, prostaglandin E2; mPGES, microsomal prostaglandin E synthase; PVN, paraventricular nucleus of the hypothalamus; RMg, raphe magnus nucleus; RPa, raphe pallidus nucleus.

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attempted to identify where in the brain this might occur using c-Fos immunocytochemistry, an indirect measure of neuronal activity; (3) tested whether PGE2 injections directly into the dorsal (DRN) and median (MnR) raphe nuclei, main sites of serotonergic neurons that project into the forebrain, are sufficient to reduce food intake, and (4) tested whether DRN-MnR COX-2 antagonism is sufficient to reduce LPS-anorexia.

2. Materials and methods

2.1. Subjects

Male Long-Evans (Experiments 1 and 2) and Sprague–Dawley (Experiments 3 and 4) rats (BW ~ 450 g), bred in our colony, were housed individually in a room maintained at 22 ± 2 °C, with a 12:12-h reversed light–dark cycle (lights on: 2300 h). Rats were housed in hanging stainless-steel wire mesh cages (33 × 18 × 20 cm) and offered ground chow (Provimi Kliba NAFAG #3433, Kaiseraugst, Switzerland) and water ad libitum, except as noted below. All procedures were approved by the Veterinary Office of the Canton of Zurich and were carried out according to the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. LPS anorexia following peripheral COX-2 antagonism

Twenty-two rats were used. One hour prior to dark onset, the rats were weighed and injected IP with saline (1 ml/kg 0.9% saline, Braun Medical, Emmenbrücke, Switzerland). Food cups were removed, filled with fresh chow and weighed. Just prior to dark onset, the rats received a second IP saline injection. Food was then returned, and intake was measured 1, 2, 4 and 23 h later. After approximately 2 weeks of adaptation to these procedures, the rats were divided in 4 groups, and food intake was measured after the following sets of injections, administered 1 hour before dark onset and just before dark onset, respectively: vehicle followed by saline (n = 5), 10 mg/kg *N*-[2-(Cyclohexyloxy)-4-nitrophenyl] methanesulfonamide (NS-398, # N-194, Sigma, Buchs, Switzerland) followed by saline (n = 5), vehicle followed by 100 μg/kg LPS (from *Escherichia coli*, serotype 0111:B4; # L-2630, Sigma) (n = 5) and NS-398 followed by LPS (n = 7). NS-398 was dissolved in a vehicle of saline plus 0.5% carboxymethyl cellulose (CMC; Bio Concept, Allschwil, Switzerland). LPS was dissolved in saline. All solutions were freshly prepared on the experimental day, with the concentrations adjusted so that injection volume was 1 ml/kg. Doses were selected on the basis of prior studies (Geary et al., 2004; Langhans et al., 1991; von Meyenburg et al., 2003a). This dose of LPS was shown to mimic the clinical features of gram-negative bacterial infection or septicemia (Lang et al., 1985; McCarthy et al., 1984; O'Reilly et al., 1988), including anorexia (Langhans et al., 1989), but to not produce apparent signs of sickness such as systemic hypotension, hyperglycemia or hyperlactacidemia or indications of endotoxin shock (Lang et al., 1985).

2.3. LPS-induced c-Fos expression following peripheral COX-2 antagonism

Twenty-four rats were adapted to the procedure described above, with the exception that food was presented 4 h after the dark onset. This was done in order to adapt the rats to food deprivation during the dark phase, so that this would not cause non-specific effects on c-Fos expression on the test day. On the test day, the same four treatments (n = 6 rats/group) as in 2.2 were administered, and 90 min after the second injections (i.e., LPS or saline) the rats were anesthetized with IP injections (1 ml/kg) of 50 mg/kg sodium pentobarbital (Nembutal, Abbott Laboratories, Abbott Park, IL, USA) and transcardially perfused with, first, 100 ml phosphate buffer (PB, 0.1 M, pH 7.4) and, second, 300 ml 4% paraformaldehyde in 0.1 M PB, both at 21 ml/min with a peristaltic pump (Cole-Parmer, Vernon Hills, IL, USA). The brains were removed, postfixed for 2 h in 4% paraformaldehyde solution at room temperature and for 2 d in 20% sucrose in 0.1 M PB at 4 °C. Six serial sets of 40 μm sections of hindbrain were cut from the obex anteriorly (i.e., from ~14.3 to ~7.3 mm posterior to bregma) according to the atlas of Paxinos and Watson. Forebrain sections were cut similarly from the optic chiasm posteriorly through the median eminence (i.e., from ~0.1 to ~4.1 mm posterior to bregma). Sections were stored in 0.1 M PB with 30% ethylene glycol and 30% glycerol at −20 °C until processing.

One set each of hindbrain and forebrain sections was processed for c-Fos expression using DAB immunocytochemistry (Asarian and Geary, 2007; Thammachaoen et al., 2008). The number of cells expressing c-Fos was quantified using Image Pro software (Version 6.0, MediaCybernetics, Silver Spring, MD, USA), as previously described (Asarian and Geary, 2007; Thammachaoen et al., 2008), in the following regions (locations are mm caudal to bregma as per Paxinos and Watson, 1986): paraventricular nucleus (PVN) (1.80 mm), central nucleus of the amygdala (CeA) (2.65 mm) and arcuate nucleus (Arc) (2.65 mm), nucleus tractus solitarii (NTS) (13.65–14.30 mm), A1 region of the ventrolateral medulla (A1) (13.65 mm), raphe magnus nucleus (RMg) (11.60–9.08 mm), MnR (8.3–7.3 mm), DRN (9.30–7.80 mm) and the raphe pallidus nucleus (RPa) which was divided into caudal RPa (cRPa) (11.30–14.08 mm), and rostral RPa (rRPa) (10.52–11.00 mm) subareas.

2.4. Anorexia following DRN/MnR administration of PGE2

To determine whether increases in PGE2 in the midbrain raphe are sufficient to inhibit eating, we injected PGE2 simultaneously into both the DRN and the MnR, where the majority of serotonin neurons express EP3 receptors. Because of the short half-life of PGE2, we trained rats to consume a highly palatable wet-mash diet at dark onset to maximize eating behavior. Eighteen rats were adapted to single caging as above for at least 1 week and then were food deprived overnight and anesthetized with 80 mg/kg ketamine HCl (Narketan 10, Chassot, Bern, Switzerland) and 4 mg/kg xylazine (Rompun, Bayer, Leverskusen, Germany). Parallel 24 gauge stainless-steel guide cannulae angled 15° toward the midline were implanted at coordinates (relative to bregma) AP + 1.2, L − 1.0 and V + 7.6 for the DRN and AP + 1.2, L − 1.0, V + 5.6 for the MnR (Hrupka and Langhans, 2001) and secured to the skull with acrylic cement and three stainless-steel screws. Stainless-steel inner stylets were inserted to maintain cannula patency. Stylets were cleaned daily for 2 days post-surgery and every other day thereafter.

Rats were allowed to recover 10 d, after which they were adapted to 20 min access to sweetened wet-mash beginning at dark cycle onset. The wet-mash diet consisted of 1 part (by weight) sweetened condensed milk, 3 parts chow and 4 parts water. Rats were food deprived 16 h prior to mash access and offered chow for 8 h daily following wet-mash. After intakes had stabilized, rats were injected at dark onset with either vehicle (n = 8; sterile saline) or PGE2 (n = 10; 100 ng/site; Sigma, P5640; prepared just prior to use) into DRN and MnR. Injection cannulas extended 0.5 mm past the guide cannulas and were left in place for ~30 s after infusions to prevent reflux. Wet-mash was offered immediately following injections. Cannula placements were verified post-mortem in sections stained with cresyl violet.

2.5. LPS anorexia following DRN/MnR COX-2 antagonism

Fifteen rats were implanted with DRN and MnR cannulas as described above. Three hours before dark onset, rats were food deprived and IP injected (1 ml/kg) with 100 μg/kg LPS or saline as a control. Just prior to dark onset, rats received simultaneous DRN and MnR infusions of either vehicle 0 (n = 7) or 1 ng NS-398 (n = 8) in

0.4 μl aCSF with 10% DMSO. Food was then returned, and intakes were measured 1, 2, 4, 7, 12 and 24 h later.

2.6. Statistical analyses

A robust statistical approach was adopted to increase statistical power (Burke, 1998; Streiner and Norman, 2006). To reduce the influence of extreme values, data were converted to standard scores using the median absolute-deviate method, and standard scores with absolute values > 1.96 (i.e., $P<0.05$) were excluded (this led to the loss of one rat from Experiment 2.2. and one from the Arc analysis in Experiment 2.3.). Data from Experiments 2.2 and 2.3 were analyzed with one-way ANOVA (Sigma Stat version 3.5, Systat Software, San Jose, CA, USA) followed up with Bonferroni–Holm tests. Logarithmic or inverse transformations were used as required to achieve normality. If this was not possible, data were analyzed with Friedman's ANOVA on ranks followed with Mann–Whitney U tests. Data from Experiments 2.4 and 2.5 were analyzed with unpaired t-tests. Parametrically analyzed data are reported as mean ± standard error of the mean (SEM), with the standard error of the difference (SED) given to indicate experiment-wide residual variability. Non-parametrically analyzed data are reported as median ± semi-interquartile range.

3. Results

3.1. LPS anorexia following peripheral COX-2 antagonism

LPS reduced cumulative food intake 2, 4 and 23 h after injection (Fig. 1).

NS-398 pre-treatment completely reversed LPS anorexia at each of these times. That is, at each time, the difference between (saline/saline vs. saline/LPS) and (NS-398/saline vs. NS-398/LPS) was statistically significant and the difference between NS-398/saline and NS-398/LPS was not. Neither saline nor NS-398 alone had any detectable effect on food intake.

3.2. LPS-induced c-Fos expression following peripheral COX-2 antagonism

LPS increased c-Fos expression in all midbrain and hindbrain regions examined, i.e., each raphe region, the NTS and A1 (Figs. 2, 3 and 4). NS-398 completely blocked LPS induced c-Fos expression in all areas except the A1, where it markedly reduced it, and the rRPa, where it had no detectable effect.

In the forebrain, LPS significantly increased c-Fos expression in the PVN and the CeA, but not in the Arc (Fig. 5).

According to the criteria above, NS-398 completely reversed c-Fos expression in the PVN, but had no effect in the CeA ($F(3, 21)=13.50$, $P<0.01$, SED = 0.2 cells/section). NS-398 alone did not affect c-Fos expression in the PVN or Arc, but did in the CeA ($F(3, 21)=13.50$, $P<0.01$, SED = 0.2 cells/section).

3.3. Anorexia following DRN/MnR administration of PGE2

Rats injected with 100 ng PGE2 into the DRN and MnR ate 35% less than vehicle-treated rats during the 20-min access to sweetened wet mash (Fig. 6; 15.0 ± 0.7 g vs. 23.1 ± 1.7 for PGE2 vs. vehicle-injected rats, $T=122$, $P<0.001$).

Vehicle-treated rats ate similar amounts of wet mash on the last pre-test baseline day and the test day (20.1 ± 1.4 vs. 23.1 ± 1.7 g for baseline vs. trial intake respectively), indicating that vehicle alone did not alter food intake.

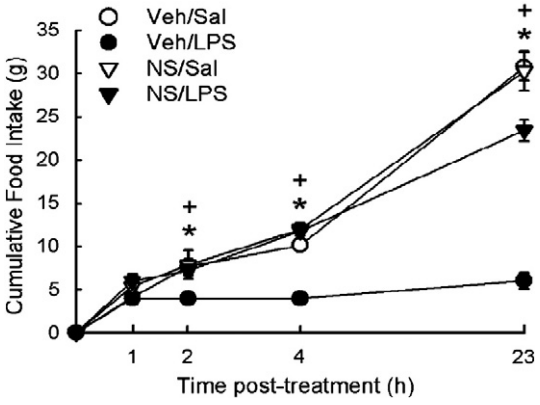

Fig. 1. Reversal of LPS anorexia by NS-398. Rats were IP injected with vehicle or 10 mg/kg NS-398 1 h before dark onset and saline or 100 μg/kg LPS at dark onset. Data are cumulative food intakes (mean ± SEM), 5–6 rats/group, $F(3, 21)=6.63$, $P<0.01$, SED = 0.08 g; $H(3)=14.70$, $P<0.01$; and $F(3, 16)=177.05$, $P<0.001$; SED = 1.40 g, for 2, 4 and 23 h, respectively. *Vehicle/LPS significantly different from vehicle/saline, Bonferroni–Holm test after significant ANOVA or Mann–Whitney *U* test after significant Friedman's ANOVA on ranks, as described in text, $P<0.05$. +(vehicle/saline–vehicle/LPS) significantly different from (NS-398/saline–NS-398/LPS), Bonferroni–Holm test after significant ANOVA or Mann–Whitney *U* test after significant Friedman's ANOVA on ranks, as described in text, $P<0.05$.

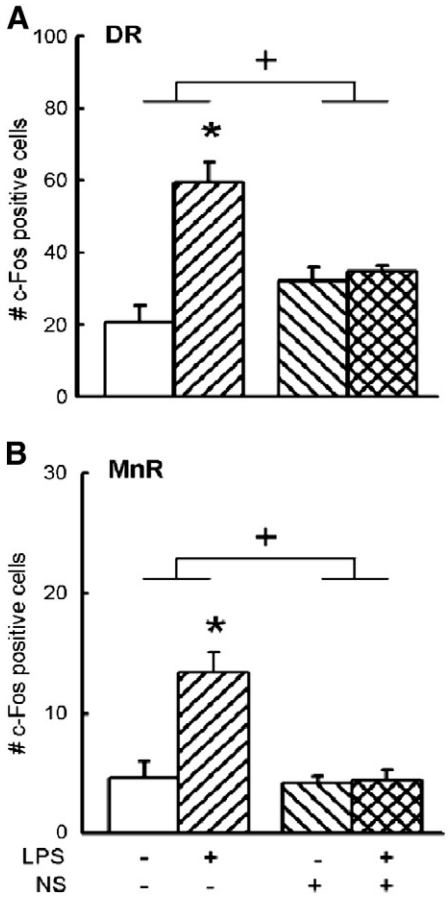

Fig. 2. Reversal of LPS-induced c-Fos expression in (A) the dorsal raphe (DRN) and (B) the median raphe (MnR) by NS-398. Rats were IP injected with vehicle or 10 mg/kg NS-398 1 h before dark onset and saline or 100 μg/kg LPS at dark onset. Data are numbers of cells/section expressing c-Fos (mean ± SEM), 5–7 rats/group $F(3, 20)=9.60$, $P<0.01$, SED = 0.1 cells, $F(3, 20)=10.90$, $P<0.01$, SED = 2.0 cells for the DRN and MnR, respectively. *Vehicle/LPS significantly different from vehicle/saline, Bonferroni–Holm test after significant ANOVA or Mann–Whitney *U* test after significant Friedman's ANOVA on ranks, as described in text, $P<0.05$. +(vehicle/saline–vehicle/LPS) significantly different from (NS-398/saline–NS-398/LPS), Bonferroni–Holm test after significant ANOVA or Mann–Whitney *U* test after significant Friedman's ANOVA on ranks, as described in text, $P<0.05$.

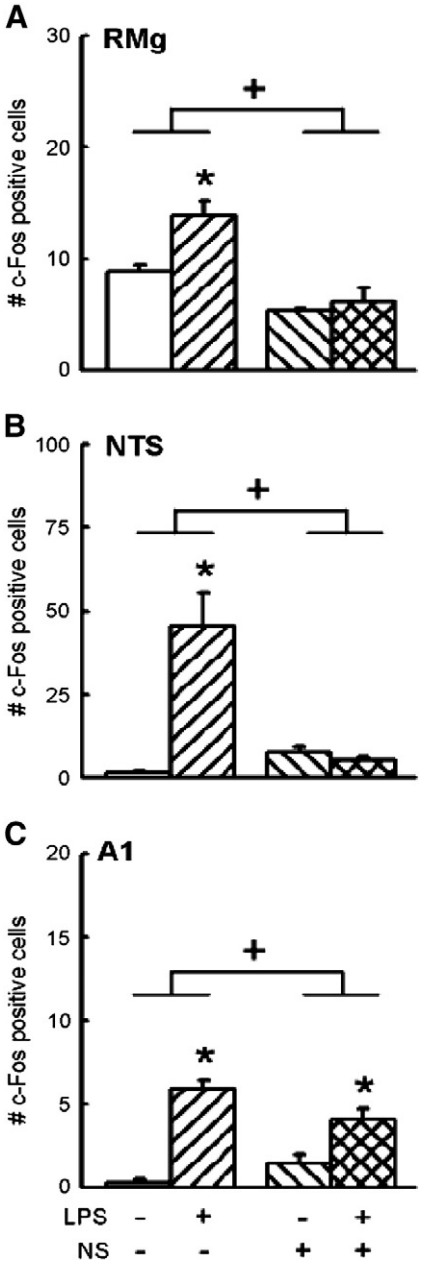

Fig. 3. Effects of NS-398 on LPS-induced c-Fos expression in (A) the raphe magnum (RMg), (B) the nucleus tractus solitarii (NTS) and (C) area 1 of the ventrolateral medulla (A1). Rats were IP injected with vehicle or 10 mg/kg NS-398 1 h before dark onset and saline or 100 μg/kg LPS at dark onset. Data are numbers of cells/section expressing c-Fos (mean ± SEM), 5–7 rats/group, $F(3, 19) = 12.90$, $P<0.01$, SED = 1.60 cells, ($H(3) = 15.46$, $P<0.01$), $F(3, 19) = 21.00$, $P<0.01$, SED = 0.1 cells, for RMg, NTS and A1, respectively. * Significantly different from control, Bonferroni–Holm test after significant ANOVA or Mann–Whitney *U* test after significant Friedman's ANOVA on ranks, as described in text, $P<0.05$. +(vehicle/saline–vehicle/LPS) significantly different from (NS-398/saline–NS-398/LPS), Bonferroni–Holm test after significant ANOVA or Mann–Whitney *U* test after significant Friedman's ANOVA on ranks, as described in text, $P<0.05$.

3.4. LPS-anorexia following DRN/MnR COX-2 antagonism

DRN/MnR injections of NS-398 significantly attenuated LPS-induced anorexia 1, 4, 7, 12 and 24 h after injection, but not 2 h after injection (Fig. 7).

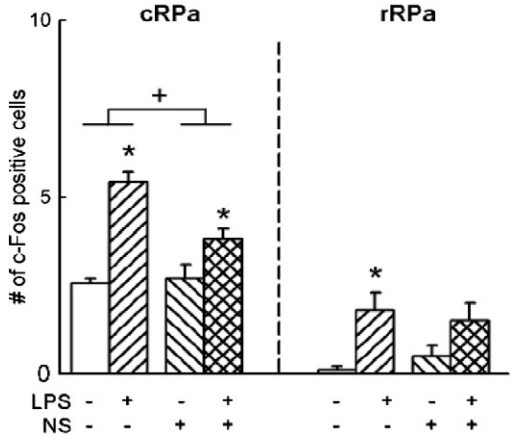

Fig. 4. Effects of NS-398 on LPS-induced c-Fos expression in the caudal raphe pallidus (cRPa) and the rostral raphe pallidus (rRPa). Rats were IP injected with vehicle or 10 mg/kg NS-398 1 h before dark onset and saline or 100 μg/kg LPS at dark onset. Data are numbers of cells/section expressing c-Fos (mean ± SEM), 5–7 rats/group, $F(3, 15) = 23.3$, $P<0.01$, SED = 0.4 cells, $F(3, 17) = 4.09$, $P<0.05$, SED = 0.6 cells for cRPa and rRPa, respectively. * Significantly different from control, Bonferroni–Holm test after significant ANOVA or Mann–Whitney *U* test after significant Friedman's ANOVA on ranks, as described in text, $P<0.05$. +(vehicle/saline–vehicle/LPS) significantly different from (NS-398/saline–NS-398/LPS), Bonferroni–Holm test after significant ANOVA or Mann–Whitney *U* test after significant Friedman's ANOVA on ranks, as described in text, $P<0.05$.

4. Discussion

Here we present behavioral and cellular evidence that advances the understanding of the role of brain PGE2 signaling in LPS anorexia. We report four principal results. First, IP injection of the specific COX-2 antagonist NS-398 1 h before IP injection of 100 μg/kg LPS eliminated LPS anorexia for the ensuing 23 h. This extends previous reports indicating that, under slightly different conditions, reductions in PGE_2 synthesis partially block LPS anorexia (Lugarini et al., 2002). Second, IP NS-398 reduced or eliminated c-Fos expression measured 90 min after LPS injection in several brain areas, ranging from the RPa in the caudal medulla to the PVN in the forebrain. These data indicate that PGE2 is necessary for some or all LPS-induced neural activation in numerous brain areas. Third, intraparenchymal injection of PGE2 into the DRN/MnR, a principal site of ascending serotonergic neurons that also contains PGE2 receptors (Nakamura et al., 2000), were sufficient to reduce food intake. Lateral hypothalamic (Baile et al., 1973) as well as intracerebroventricular (ICV) (Levine and Morley, 1981) injections of PGE2 have been reported to reduce eating in rats, but this is the first evidence we know implicating hindbrain PGE2 in the control of eating. Fourth, injection of NS-398 into the DRN/MnR significantly reduced IP LPS anorexia. This result suggests that increased PGE2 in the DRN/MnR is required for IP LPS anorexia.

IP NS-398 markedly reduced LPS-induced c-Fos expression measured 90 min post-LPS injection. LPS-induced c-Fos expression was statistically eliminated by NS-398 in the DRN, MnR, RMg, cRPa, NTS, and PVN. C-Fos expression reflects electrophysiological activity about an hour earlier, i.e., in our experiment soon after LPS administration. Thus, our data suggest that much of the initial neuronal activation induced by IP LPS in these areas requires PGE2 signaling. Furthermore, because anorexia began to develop around the same time, some or all of these areas may mediate the onset of LPS anorexia.

We hypothesized that the midbrain raphe nuclei, i.e., the DRN and MnR, are likely to be sites where PGE2 that is released into the parenchymal interstitial fluid in response to activation of inducible COX-2 in the blood–brain barrier endothelial cells by LPS or immune mediators acts on serotonin neurons to initiate neural signals ultimately leading to anorexia (Asarian and Langhans, 2010). This

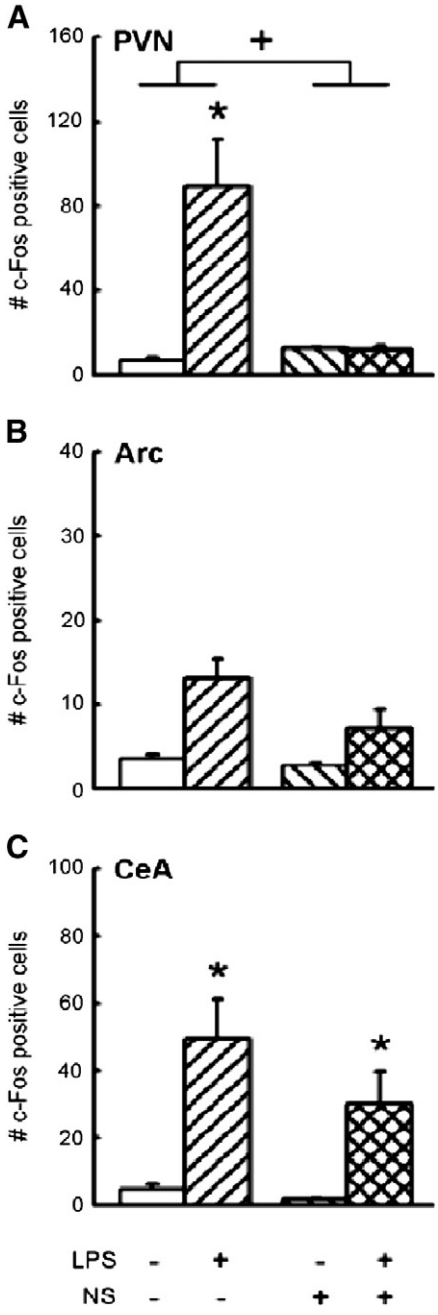

Fig. 5. Effects of NS-398 on LPS-induced c-Fos expression in (A) the paraventricular nucleus of the hypothalamus (PVN), (B) the arcuate nucleus (Arc) and the central nucleus of the amygdala (CeA). Rats were IP injected with vehicle or 10 mg/kg NS-398 1 h before dark onset and saline or 100 μg/kg LPS at dark onset. Data are numbers of cells/section expressing c-Fos (mean ± SEM), 5–7 rats/group, $F(3,22) = 16.20$, $P<0.01$, SED = 0.2 cells/section, $H(3) = 5.50$, $P>0.05$, $F(3,21) = 13.50$, $P<0.01$, SED = 0.2 cells/section, in the PVN, Arc and CeA, respectively. * Significantly different from control, Bonferroni–Holm test after significant ANOVA or Mann–Whitney *U* test after significant Friedman's ANOVA on ranks, as described in text, $P<0.05$. +(vehicle/saline–vehicle/LPS) significantly different from (NS-398/saline–NS-398/LPS), Bonferroni–Holm test after significant ANOVA or Mann–Whitney *U* test after significant Friedman's ANOVA on ranks, as described in text, $P<0.05$.

hypothesis rests on the anatomical position of the midbrain raphe as the principal source of ascending serotonergic projections to forebrain areas controlling eating, and on the extensive evidence that serotonin neural signaling is necessary for IP LPS anorexia. This evidence includes reports that midbrain raphe serotonin neurons express EP3 receptors (Nakamura et al., 2000) and are activated by PGE2 (Ericsson et al., 1997; Nakamura et al., 2001), that administration of serotonin 1A receptor agonist 8-OH-DPAT attenuated IP and ICV LPS anorexia in

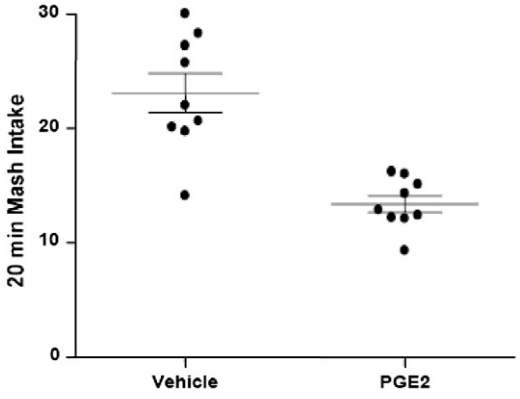

Fig. 6. Administration of PGE2 into the dorsal raphe (DRN) and median raphe (MnR) reduces food intake. Rats were food deprived for 16 h and infused intracranially with 100 ng PGE2 during the early dark phase. Rats were allowed 20 min access to sweetened mash following PGE2 injections. Data are cumulative food intakes (mean ± SEM), 9 rats/group, $t(16) = 0.4$, $P>0.05$. * Significantly different from control, $T = 122$, $P<0.001$.

rats (Hrupka and Langhans, 2001; von Meyenburg et al., 2003b), that DRN neurons release serotonin after LPS treatment (Hollis et al., 2006), and, finally, that under the same conditions as tested here, antagonism of serotonin 2C receptors reversed both LPS-induced anorexia and midbrain raphe c-Fos expression (Kopf et al., 2010). Our findings that, first, local PGE2 injections into the midbrain raphe inhibit eating and, second, that inhibition of PGE2 signaling in the midbrain raphe reduces IP LPS anorexia add to these lines of evidence implicating midbrain raphe PGE2 signaling in LPS anorexia. Our data fall short of proof of this hypothesis, however, because we did not exclude the possibility that our local injections spread sufficiently to affected areas outside the midbrain raphe. Furthermore, our experiments did not establish that our manipulations of midbrain raphe PGE2 signaling affected eating and LPS anorexia by acting directly on serotonergic neurons.

Intrahypothalamic and ICV injections of PGE1, PGE2, and PGF2α were reported to reduce food intake more than 35 years ago (Baile et al., 1973; Levine and Morley, 1981). This is the first such report for midbrain PGE2 injection. Furthermore, the dose of PGE2 that we

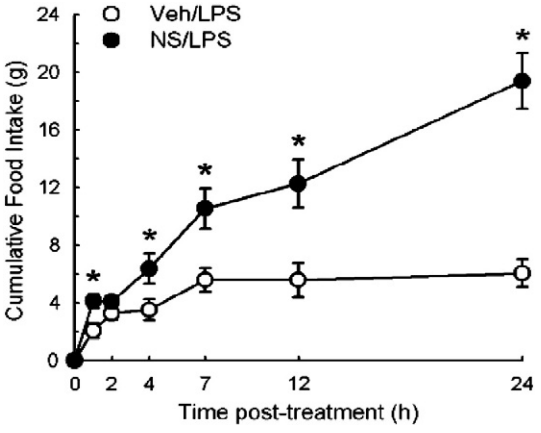

Fig. 7. Reversal of LPS anorexia by NS-398 administered into the dorsal/median raphe (DRN/MnR). Rats were food deprived for 3 h before dark and at the same time injected IP with 100 μg/kg LPS or saline and at dark onset infused into the DRN/MnR with 0 or 1 ng NS-398. Food was returned and food intake was measured for 1, 2, 4, 7, 12 and 24 h. Data are cumulative food intakes (mean ± SEM), 8–9 rats/group, $t(12) = 12.9$, $P<0.01$, SED = 0.7 g; $t(9) = 1.41$, $P>0.05$, SED = 0.6 g, $t(11) = 2.25$, $P<0.04$, SED = 1.3 g; $t(12) = 2.9$, $P<0.01$, SED = 0.7 g; $t(13) = 3.15$, $P<0.007$, SED = 2.12 and $t(11) = 5.15$, $P<0.003$, SED = 2.6 g, for 1, 2, 4, 7, 12 and 24 h, respectively. * Significantly different from control, Bonferroni–Holm test after significant ANOVA or Mann–Whitney *U* test after significant Friedman's ANOVA on ranks, as described in text, $P<0.05$.

found effective when injected into the DRN/MnR (100 ng/site), is smaller than previously found effective in the forebrain, which further supports the midbrain raphe as a potential site of action for prostaglandin in the control of eating. Whether midbrain PGE2 injection inhibits eating via an aversive mechanism is not clear. Although we observed no anomalous behaviors during the injections, we did not formally monitor behaviors other than eating, or measure body temperature. Baile et al. (1973) observed no signs of behavioral depression effects with larger doses of prostaglandin.

Similar to the effects of LPS and NS-398 in the DRN and MnR, LPS potently increased c-Fos expression in the PVN, and this was completely reversed by NS-398. Our data extend numerous previous reports that LPS induces c-Fos expression in the PVN (Lacroix and Rivest, 1997; Laflamme et al., 1999; Rivest and Laflamme, 1995; Valles et al., 2005; Wan et al., 1994). The LPS doses, routes of administration, and times at which c-Fos was measured vary widely across these studies, suggesting that the PVN is an important site for both the initial and subsequent phases of the APR. PGE2 may be involved in mediating LPS's effect in the PVN as well. Zhang et al. (2003), for example, reported that IP administration of SC-236, another specific COX-2 antagonist, reversed LPS induced c-Fos expression in the PVN 4 h following intravenous injection of 4 μg/kg LPS. Although there is no direct evidence implicating the PVN in illness anorexia, it seems likely that it is given its important role in the control of eating. As well, several reports indicate that PGE2 affects signaling in PVN corticotrophin-releasing hormone (CRH) neurons (Ericsson et al., 1997; Lacroix et al., 1996; Zhang and Rivest, 1999), and CRH appears to play a role in the control of eating independent of its role in the hypothalamic–pituitary–adrenal axis (Grill et al., 2000; Lu et al., 2003).

LPS-induced c-Fos expression was significantly reduced, but not eliminated, by NS-398 in the cRPa and A1, suggesting that PGE2 contributes to the initial neuronal activation induced by IP LPS in these areas. It has been reported that LPS and other inflammatory mediators can affect the RPa neurons (Gemma et al., 1991; Nakamura et al., 2002). These studies, however, used larger LPS doses (250 μg and 1 mg/kg) and measured c-Fos at later time points (3 and 4 h after LPS administration) (Laflamme et al., 1999; Valles et al., 2005) than we did here. Therefore, our data therefore extend previous reports by demonstrating that a low dose of LPS induces c-Fos in RPa near the time when LPS anorexia develops.

Because anorexia and fever develop with about the same latency, it is likely that some or all of the areas in which LPS and NS-398 affected c-Fos expression here are also involved in mediating LPS's febrile effects. Our RPa data are especially interesting in this connection. Nakamura et al. (2002) reported that the number of cells expressing c-Fos in the rRPa, but not in the cRPa, was increased 1 h after injection of PGE2 into the lateral ventricle or preoptic area (POA) in urethane-anesthetized rats. In contrast, using the same rostral–caudal definition, we found that IP LPS increased c-Fos expression in the cRPa as well as in the rRPa and that IP NS-398 significantly reduced cRPa c-Fos, but not rRPa c-Fos. Taken together, these data suggest, first, that IP LPS activates the cRPa by a mechanism that involves, at least in part, PGE2 signaling that does not arise in the POA or other sites near the lateral ventricles, and, second, that the PGE2-dependent input to the rRPa from the POA may not be activated by IP LPS. In so far as IP injection of the moderate LPS dose that we used is considered to be a valid model of illness, the latter conclusion suggests that the pathophysiological relevance of POA PGE2 injections deserves further research. It is also important to note in this connection that we did not investigate c-Fos expression in many brain areas that have been implicated in fever or visceral elements of the APR, in particular the preoptic nucleus and the lateral hypothalamic area.

In contrast to the PVN results, LPS alone had no significant effect on c-Fos expression in the Arc and, although LPS increased c-Fos in the CeA, NS-398 did not affect this response. LPS induced increases in c-Fos expression in the CeA have been reported before under various other conditions (Dallaporta et al., 2007; Lacroix and Rivest, 1997; Laflamme et al., 1999; Marvel et al., 2004; Rivest and Laflamme, 1995; Valles et al., 2005; Wan et al., 1993, 1994). Our data extend these reports and, in addition, suggest that PGE2 signaling is not a necessary part of the initial LPS-induced neuronal activation in the CeA. Our failure to detect an increase in c-Fos in the Arc appears inconsistent with several previous studies (Marvel et al., 2004; Wan et al., 1993), perhaps because of differences in LPS dose or time of sampling. For example, in one study (Lacroix and Rivest, 1997), the higher IP dose of 1 mg/kg LPS did not induced c-Fos mRNA expression 2 h after injection, but did so 4 h after injection.

IP LPS also increased c-Fos in two brainstem areas. The increase that we observed in the NTS is consistent with previous reports (Lacroix and Rivest, 1997; Laflamme et al., 1999; Rivest and Laflamme, 1995; Sagar et al., 1995; Valles et al., 2005; Wan et al., 1994). In addition, the reduction in this effect by NS-398 is consistent with the report that IP LPS produced less c-Fos in the NTS of mPGES-1 knockout mice (Dallaporta et al., 2007). We also observed that IP LPS increased c-Fos in the A1 area and that this was reduced by NS-398, suggesting that noradrenergic (NA)/CA pathways may be part of the mechanism mediating the COX-2 effect on LPS anorexia. Consistent with this, Lacroix and Rivest (1997) reported that indomethacin decreased the number of tyrosine hydroxylase-immunoreactive cells that express LPS-induced c-Fos mRNA.

Taken together with previous reports, our data suggest new perspectives concerning the neural network mediating LPS anorexia. In particular, we propose that peripheral immune signaling activates inducible COX-2 in endothelial cells in the midbrain raphe, leading to PGE2 release, stimulation of neuronal EP3 receptors, and activation of the neural network mediating anorexia. Furthermore, we hypothesize that many or most of these neurons are serotonergic. Finally, because the PVN is an important target of ascending serotonergic fibers from the midbrain raphe and because the c-Fos effects we measured in the DRN/MnR and in the PVN were quite similar and distinct from those in other forebrain sites, it seems likely that the PVN is an important forebrain node in this network. We propose this network as a working model for future studies of LPS anorexia.

The complexity of neuro-immune interactions presents a great impediment to understanding the mechanisms of LPS-anorexia (Asarian and Langhans, 2010). Although we previously identified serotonin neurons as crucial elements in LPS anorexia (von Meyenburg et al., 2003b) and the present results indicate that PGE2 signaling in the DRN/MnR is necessary for LPS anorexia, we are not yet able to conclude that these serotonin neurons are directly activated by PGE2. This would require recording of serotonergic neurotransmission, for example with microdialysis, during DR/MnR PGE2 stimulation. Furthermore, the presence of EP3 receptors in the DRN and MnR and the ability of PGE2 to reduce food intake when microinjected into the DRN/MnR suggest that locally synthesized prostaglandin acts on these receptors to mediate LPS-induced anorexia, but does not prove it. This needs to be verified by local administration of a selective EP3 antagonist with appropriate controls to assess the spread of the infusate. Our studies indicate that during the initiation of LPS anorexia the midbrain raphe and its projections to forebrain regions such as PVN, Arc and CeA might play a very important role. More generally, our data provide further evidence supporting the concept that infection anorexia arises from altered neural processing within the same networks that mediate normal eating.

Acknowledgments

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