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# Inflammation-induced anorexia and fever are elicited by distinct prostaglandin dependent mechanisms, whereas conditioned taste aversion is prostaglandin independent

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ABSTRACT

Systemic inflammation evokes an array of brain-mediated responses including fever, anorexia and taste aversion. Both fever and anorexia are prostaglandin dependent but it has been unclear if the cell-type that synthesizes the critical prostaglandins is the same. Here we show that pharmacological inhibition or genetic deletion of cyclooxygenase (COX)-2, but not of COX-1, attenuates inflammation-induced anorexia. Mice with deletions of COX-2 selectively in brain endothelial cells displayed attenuated fever, as demonstrated previously, but intact anorexia in response to peripherally injected lipopolysaccharide (10 μg/kg). Whereas intracerebroventricular injection of a cyclooxygenase inhibitor markedly reduced anorexia, deletion of COX-2 selectively in neural cells, in myeloid cells or in both brain endothelial and neural cells had no effect on LPS-induced anorexia. In addition, COX-2 in myeloid and neural cells was dispensable for the fever response. Inflammation-induced conditioned taste aversion did not involve prostaglandin signaling at all. These findings collectively show that anorexia, fever and taste aversion are triggered by distinct routes of immune-to-brain signaling.

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## 1. Introduction

Anorexia, i.e. loss of appetite, and taste aversion are common and debilitating symptoms that occur as consequence of acute and chronic inflammatory disease (Saper et al., 2012). While it is well established that inflammation-induced anorexia is prostaglandin (PG) dependent, as demonstrated by the amelioration of the food intake by cyclooxygenase (COX) inhibitors (Baile et al., 1981; Langhans et al., 1989; Lugarini et al., 2002; Swiergiel and Dunn, 2002), the cellular source of the prostaglandins that evoke anorexia has not been identified. This is in contrast to fever, another cardinal symptom of the inflammatory response. As demonstrated in genetically modified mice, fever is dependent on $PGE_2$ synthesized in brain endothelial cells (Engstrom et al., 2012; Wilhelms et al., 2014), and its subsequent binding to dedicated receptors on thermosensory neurons in the preoptic hypothalamus (Lazarus et al., 2007). However, prostaglandins are induced in several other cell-types upon inflammation and the induction in different cell-types may mediate distinct symptoms and has even been reported to elicit opposing effects (An et al., 2014; Serrats et al., 2010). In the same vein, neural prostaglandin synthesis is critical for the hyperalgesia induced by a local inflammation but dispensable for fever (Vardeh et al., 2009), whereas brain endothelial prostaglandins synthesis is critical for immune-induced fever but dispensable for the reduction in locomotor activity triggered by the same stimulus (Wilhelms et al., 2014).

We here examined the cellular source for prostaglandins eliciting inflammation-induced anorexia by using mice with cell-type specific deletions of COX-2, the COX isoform that is responsible for eliciting both fever (Cao et al., 1997; Li et al., 1999) and anorexia (Lugarini et al., 2002; Swiergiel and Dunn, 2002). To directly test if anorexia and fever are driven by COX-2 in the same cell-types we also monitored fever induced by LPS of the same type and dose (10 μg/kg) as used in the anorexia experiments. Furthermore, we included analysis of inflammation-induced conditioned taste aversion (CTA) since this symptom is closely related to anorexia but has been shown, at least partly, to involve distinct mechanisms (Bauer et al., 1995; Kopf et al., 2011).

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## 2. Materials and methods

### 2.1. Animals

All experiments followed international and national guidelines, and were approved by the animal care and use committee in Linköping. Mice were kept on a 12–12 h light-dark cycle. Food and water were provided ad libitum if not stated otherwise. Adult mice (older than 8 weeks), of both sexes were used. During the experimental period, all mice were housed individually. For fever experiments, mice were kept at near-thermoneutral ambient temperature of 28 °C ± 1 °C (humidity 42–49%). All other experiments were performed at room temperature (21 °C ± 1 °C, humidity 40–43%).

C57B/6 wild type (WT) mice used for the pharmacological studies were purchased from Scanbur (Karlslunde, Denmark). COX-1 KO (Langenbach et al., 1995) and COX-2 KO (Morham et al., 1995) were purchased from Taconic Biosciences Inc (Ejby, Denmark), and were on a mixed B6;129P2 background. Mice that had the gene encoding COX-2 flanked by loxP sites (floxed) (Ishikawa and Herschman, 2006) were crossed with mice expressing Cre under the control of the nestin promoter (MGI: J:57315, The Jackson Laboratory, Bar Harbor, ME), the LysM promoter (MGI: J:67924, Jackson Laboratories) or Slco1c1 promoter (Ridder et al., 2011) to obtain mice with deletion of COX-2 in the neural cells (Cox2ΔNes), myeloid cells (Cox2ΔLysM), or brain endothelial cells (Cox2ΔbEnd), respectively. Homozygous floxed ($Cox2^{fl/fl}$) but Cre negative littermates were used as controls (WT). C57B/6 WT mice implanted with cannulas into the brain ventricular system were purchased from Jackson Laboratories (Bar Harbor, ME). In general, littermates were used as controls except for the experiment with COX-2 knockouts in which mice with the same genetic background but not from the same colony were used. In all experiments the groups were balanced regarding sex and age.

To induce Cre-recombinase activity in mice with a Slco1c1-$CreER^{T2}$ construct, 100 μL tamoxifen solution (1 mg tamoxifen dissolved in a 1:10 mixture of ethanol and sunflower seed oil) was administered intraperitoneally (i.p.), twice a day for 5 consecutive days.

### 2.2. Drugs

To induce anorexia, 100 μL LPS (O55:B5, Sigma Aldrich, St. Louis, MO; 10 μg/kg) was given i.p. The unspecific COX-inhibitor indomethacin (Confortid; Alpharma, Langenfeld, Germany; 5 mg/kg) and the COX-2 specific inhibitor parecoxib (Dynastat; Pfizer, New York, NY; 10 mg/kg) were given i.p. at a volume of 100 μL. These doses were selected from previous studies (Nilsberth et al., 2009; Ruud et al., 2013a) and tested by titration to robustly inhibit anorexia induced by the given dose of LPS. Indomethacin (Confortid) was also administered intracerebroventricularly (i.c.v.; 15 μg in 3 μL). The COX-1 specific inhibitor SC-560 (Cayman chemicals, Ann Arbor, MI; 30 mg/kg in 300 μL) was given by gavage. The selected dose has previously been show to robustly inhibit COX-1 in a food intake paradigm (Ruud et al., 2013a). Indomethacin, parecoxib and LPS were diluted in saline, whereas SC-560 was diluted in a mixture of methylcellulose and tween 80. Due to the problem with tolerance against LPS, each animal was only given LPS once.

### 2.3. Fever experiments

One week prior to the recordings, temperature transmitters (model TA11TAF10, Data Sciences International, New Brighton, MN) were inserted into the abdominal cavity of the mice under brief gas anesthesia (isofluoran, 1%). Temgesic was given peri-operatively. Basal core temperature was measured 24 h prior to experimental onset. About 2–3 h after lights on, mice were given a single i.p. injection of LPS or saline and core body temperature was measured for 12 h. Experiments were performed during daytime in order to minimize activity-related changes in body temperature. Fever was defined as any prolonged LPS-induced increase in body temperature (i.e. when LPS-treated mice had a body temperature that was statistically significantly higher than that of NaCl-treated mice).

### 2.4. Food intake experiments

Mice were single housed for a minimum of 5 d prior to food intake measurements. All food intake measurements were performed during the active period of the mice, i.e. during the dark period. On the test day, food was withdrawn and mice were given a single injection of either LPS or saline 1 h before onset of the dark period (7 p.m.). At dark period onset, pre-weighed food was given, and the weight of the food was measured again at 4, 7 and/or 13 h after injection. Visible food spillage in the cage was measured and accounted for. If food spillage was detected after experimental endpoint, the data from that particular animal was excluded. Pretreatment with indomethacin or SC-560 was done once, 1 h before LPS or saline injection. Parecoxib was given 30 min before LPS or saline and an additional dose was given after 4.5 h. The timing for administration of pretreatment was chosen in concordance with studies in which the drugs have been demonstrated to be efficient in inhibiting a LPS response (Fritz et al., 2016; Nilsberth et al., 2009).

For experiments using i.c.v. administration of indomethacin, mice were single housed 5 d before experimental onset. On experimental day, food was withdrawn 2 h prior to dark period onset. Four hours after food withdrawal, 3 μL indomethacin (15 μg) was injected with a Hamilton syringe into the i.c.v. cannula under brief gas anesthesia (isofluoran, 1%), and LPS was administered i.p. simultaneously. Ten minutes after injections, pre-weighed food was reintroduced and food intake was measured after 1 h by weighing the food tray.

### 2.5. Conditioned taste aversion experiments

All behavioral testing was conducted during the dark phase. Mice were separated and housed one and one a minimum of 5 d prior to experimental onset. Days 1–7, mice were habituated to water deprivation for 4 h a day. Day 8 (conditioning day), mice were water deprived for 4 h, then given access to a saccharin solution (0.15%) for 1 h, and immediately thereafter given an injection of either LPS or saline. Days 9–10 mice were only exposed to water deprivation. At the test day (Day 11) mice were water deprived for 4 h and then given saccharin solution for 1 h after which saccharin intake was measured. Pretreatment with SC-560 and parecoxib was given 1 h before LPS injection on the conditioning day (Day 8), i.e. at the same time mice got access to saccharin. The timing for administration of these inhibitors is in concordance with the timing of administration in the food intake studies.

### 2.6. Statistics

Results are presented as mean ± SEM. Anorexia and fever were analyzed with a 2-way repeated measures ANOVA, followed by a post hoc analysis using Tukey's (anorexia) or Holm-Sidak's (fever) multiple comparisons test. Mean fever and saccharin intake were analyzed with a 2-way ANOVA, followed by a post hoc analysis using Tukey's multiple comparisons test. $P < 0.05$ was considered statistically significant.

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