

Distinct Roles for Cyclooxygenases 1 and 2 in Interleukin-1-Induced Behavioral Changes

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ABSTRACT

Interleukin-1 (IL-1) induces hypophagia, which can be reduced by cyclooxygenase (COX) inhibitors. Earlier studies with COX knockout (COXko) mice suggested that COX2 was more important for hypophagia than COX1. However, behavioral responses occur long before COX2 is induced. Hypophagia was assessed in mice by measuring the intake of sweetened milk in a brief period. The intake was reduced within 30 min after intraperitoneal injection of IL-1 β and was depressed for about 2 h. When milk intake was measured 30 to 40 min after IL-1 β , COX1ko mice showed an attenuated response, whereas COX2ko mice responded more like wild-type animals. By contrast, 90 to 120 min after IL-1 β COX1ko mice responded normally, whereas COX2ko mice showed only small responses. The COX2-selective inhibitor, celecoxib, failed to alter the re-

sponse to IL-1 β 30 min after administration, but low doses antagonized the effects of IL-1 β at 90 to 120 min. The COX1-selective inhibitor, SC560, attenuated both the early and late responses, but a larger effect at 30 min than at 90 min suggested a role for COX1 at the earlier time. These results suggest that shortly after IL-1 β administration, COX1 is the major enzyme involved in the reduction of milk intake, whereas at later times COX2 is more important, paralleling its induction. Celecoxib also attenuated the milk intake response observed 2 h after lipopolysaccharide (LPS), and the reductions of food pellet intake and body weight induced by IL-1 β and LPS in the subsequent 24 h, suggesting that the role of COX2 may be more significant biologically than that of COX1.

Interleukin-1 β (IL-1), a cytokine secreted during inflammatory processes, induces substantial changes in behavior, including a depression in feeding (McCarthy et al., 1985; Dantzer et al., 2001). Many factors have been implicated in the control of feeding, but in our previous studies, of the many antagonists tested, only inhibitors of cyclooxygenase (COX), a key enzyme in the synthetic pathways for prostaglandins, prostacyclins, and thromboxanes, significantly modified IL-1-induced hypophagia (Hellerstein et al., 1989; Langhans et al., 1993; Swiergiel et al., 1997a; Swiergiel and Dunn, 2001; Dunn and Swiergiel, 2000). The existence of two different COX isozymes, COX1 and COX2, is now well established (Frölich, 1997). COX1 is constitutively and ubiquitously expressed, whereas COX2 is constitutively expressed to a limited extent only in certain cells, including neurons in the brain (Breder et al., 1995). COX2 is highly inducible and is thought to play an important role in inflammatory responses. Expression of COX2 is greatly increased in many

tissues when the immune system is stimulated or following administration of IL-1 or a bacterial endotoxin (lipopolysaccharide, LPS). In the brain, COX2 is induced primarily in cerebral endothelial cells and perhaps in microglia (Cao et al., 1996; Elmquist et al., 1997). Because eicosanoids synthesized in the endothelia may readily penetrate the brain, induced COX2 is well positioned to affect behavior, including feeding. However, although our previous studies with selective COX1 and COX2 inhibitors tended to implicate COX2 as the important isoform, a role for COX1 could not be excluded. An important discrepancy exists between the relatively slow induction of COX2 and the behavioral responses to IL-1 that can be observed relatively soon after IL-1 administration. Thus, we compared the effects of selective COX inhibitors at different times after IL-1 and in mice lacking functional genes for either COX1 or COX2.

Materials and Methods

Animals. Six-week-old CD-1 male mice (VAF Plus colony R16) were purchased from Charles River (Raleigh-Durham, NC). Six-week-old COX1 (B6.129P2-*Ptgs1^{tm1}*) and COX2 (B6.129P2-*Ptgs2^{tm1}*) knockout male mice were obtained from Taconic Farms (Germantown, NY), which breeds previously created COX1 and COX2 knock-

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ABBREVIATIONS: IL-1, interleukin-1 β ; mL-1 β , mouse IL-1 β ; COX, cyclooxygenase; LPS, lipopolysaccharide; SC560, 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethylpyrazole; DMSO, dimethyl sulfoxide; ANOVA, analysis of variance; COXko, COX knockout; NS-398, *N*-[2-cyclohexyloxy-4-nitrophenyl]methanesulfonamide.

out mice (Morham et al., 1995; Langenbach et al., 1999). Genotyping using polymerase chain reaction was performed by the breeder. Mice were housed singly in plastic cages with wood shaving bedding under a 12-h light-dark cycle with lights on at 6:00 AM and at 22–23°C room temperature in the Association for Assessment and Accreditation of Laboratory Animal Care-accredited facilities. All animals were given free access to water and Purina chow. All procedures were approved by the Louisiana State University Health Sciences Center Animal Care Committee and conformed to National Institutes of Health guidelines.

Sweetened Milk Intake. Intake of food pellets and sweetened condensed milk diluted with three parts water was assessed as described previously (Swiergiel et al., 1997a). Mice were habituated to drink milk through 20-ml glass bottles fitted with metal spouts once a day for 30 min. When the animals consistently drank at least 1.5 g of milk during a session, the experiments were commenced. At about 8:00 AM, the remaining solid food was removed from the cages and weighed. The weighed milk bottles were placed in the cages at approximately 11:00 AM for either 10 or 30 min, then removed and reweighed to determine the amount consumed. Consumption of solid food was assessed by placing two fresh and firm food pellets in the cage of each mouse immediately after completing a daily experiment. The next morning the remaining food pellets were removed and weighed. Changes in body weight were followed by weighing the animals at least once a week and on all experimental days.

Materials. Recombinant mouse interleukin-1 β (mIL-1 β) was purchased from R & D Systems (Minneapolis, MN) and *Escherichia coli* LPS was obtained from Sigma-Aldrich (St. Louis, MO; L3755, serotype 026:B6). mIL-1 β (100 ng/mouse) and LPS (1 μ g/mouse) were dissolved in sterile pyrogen-free 0.9% sodium chloride such that the total dose for each mouse was contained in 0.1 ml, which was injected i.p. Celecoxib and SC560 (generous donations from G. D. Searle, Skokie, IL) were dissolved in dimethyl sulfoxide (DMSO) and diluted with saline to a final concentration of 20% (v/v) DMSO.

Experimental Procedures. In a preliminary experiment to determine the time course of the behavioral response to IL-1, the milk bottle was placed in the cage immediately, 30, 60, 90, 120, 180, or 240 min after IL-1 and removed 30 min later. A 30-min period was used for the assessment of milk intake 90 min after IL-1 or 120 min after LPS. When intake was assessed 30 min after IL-1 administration, the period for which the milk was accessible was reduced to 10 min, so that the behavioral assessment was completed long before the presumed appearance of induced COX2. This resulted in a slightly lower milk intake at the 30-min time point, although in previous experiments most of the milk consumption occurred in the first 10 min of the 30-min period. The COX inhibitors or DMSO vehicle were administered s.c. 30 min before injection of IL-1 or LPS. The mice received multiple injections of IL-1 but were rotated within the experimental groups, so that no mouse received IL-1 in consecutive behavioral tests, and rarely twice in the same week. Results of numerous previous experiments have indicated that if injections of IL-1 were spaced 2 or more days apart, the hypophagic response to

IL-1 was not influenced by the earlier treatment. LPS has been shown to have an effect on subsequent administrations, so each mouse received LPS only once. The experiments with SC560 were performed on 3 separate days with mice rotated in a manner such that each mouse received each dose of SC560.

Data Analysis. Multifactorial analysis of variance (ANOVA) was performed using SuperAnova (Abacus Concepts, Inc., Berkeley, CA). The factors were treatment (saline and IL-1 or LPS), genotype (COX1ko and COX2ko), or drug (vehicle, celecoxib, or SC560) and time after the treatment. Post hoc comparisons were made using Fisher's protected least significant difference test. All data are reported as mean \pm standard error of the mean.

Results

Time Course of Feeding Responses to mIL-1 β . The time course of milk drinking response was assessed by placing the bottles of sweetened milk in the cage for 30 min at various times after i.p. injection of saline or mIL-1 β (Fig. 1). Repeated measures ANOVA revealed significant effects of IL-1 ($F_{1,169} = 37, p < 0.001$) and time ($F_{6,169} = 3.06, p < 0.01$), and a significant IL-1 \times time interaction ($F_{6,169} = 3.60, p < 0.01$). Reductions of milk intake occurred within the first 30 min after injection. Milk intake remained low from about 30 to 120 min after IL-1 β , and returned to normal within 3 h. The animals begin drinking the milk almost immediately after the bottles were placed in the cage; thus, a response to IL-1 β occurred within a few minutes after IL-1 β injection. In a 30-min period, most of the milk was consumed within the first 10 min; shorter drinking bouts occurred later. In additional experiments (data not shown), access to milk was restricted to 10 min, and milk intake was measured at various times after mIL-1 β injection. Milk intake was not significantly affected in the period 5 to 15 min after injection of IL-1; however, in the period 15 to 25 min after IL-1, the milk consumption was significantly depressed.

Time-Dependent Behavioral Effects of mIL-1 β in COX1 and COX2 Knockout Mice. Figure 2 shows the milk intake responses to mIL-1 β in COX1ko and COX2ko mice at 30 and 90 min after injection. When milk intake was assessed 30 min after injection (top panel), mIL-1 β decreased drinking significantly ($F_{1,20} = 13.1, p < 0.01$) but only in COX2ko mice ($p < 0.001$), not in COX1ko mice. A significant effect of genotype ($F_{1,20} = 15.7, p < 0.0001$) and a significant interaction between genotype and IL-1 ($F_{1,20} = 8.07, p < 0.001$) suggested that COX1 was important for this response. Administration of mIL-1 β also decreased drinking 90 min after injection ($F_{1,20} = 43, p < 0.0001$). However, as reported

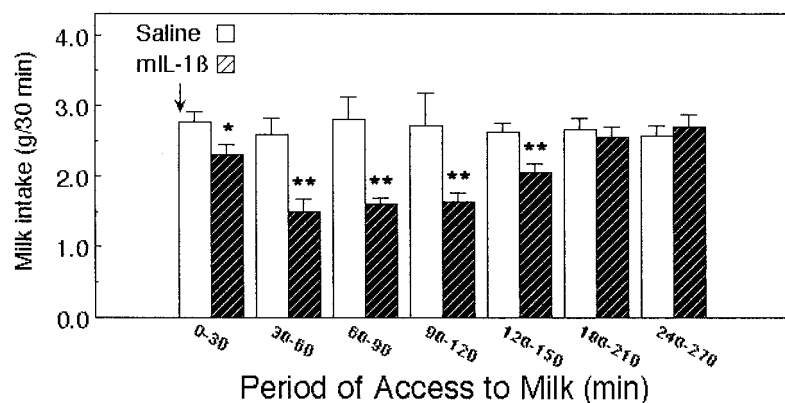


Fig. 1. The time course of the effects of mIL-1 β administration on sweetened milk intake by CD-1 mice. A bottle containing sweetened milk was placed in the cage for 30 min at various times after administration of saline or mIL-1 β (100 ng/mouse i.p.). $N = 4$ to 26 per time point (results were pooled from six separate experiments). Significantly different from the saline control group (*, $p < 0.05$; **, $p < 0.01$).

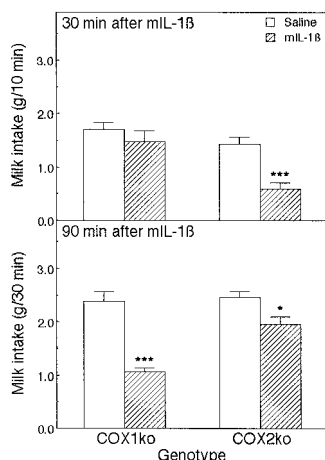


Fig. 2. The effect of mIL-1 β on sweetened milk intake in COX1 and COX2 knockout mice. Milk was presented 30 min after administration of saline or mIL-1 β (100 ng/mouse i.p.) for 10 min (top panel) or 90 min after mIL-1 β for 30 min (bottom panel). For COX1ko, $N = 5$; for COX2ko, $N = 7$. The 90-min results were reported previously (Swiergiel and Dunn, 2001). Significantly different from the saline control group (*, $p < 0.05$; ***, $p < 0.001$).

previously (Swiergiel and Dunn, 2001), this late response was more profound in COX1ko than in COX2ko mice (Fig. 2, bottom panel). The effect of genotype was statistically significant ($F_{1,20} = 12.0$, $p < 0.01$), and there was a significant interaction between genotype and IL-1 ($F_{1,20} = 8.91$, $p < 0.01$). These results indicate that the behavioral response observed 90 min after IL-1 β injection was impaired in COX2ko mice.

COX1ko and COX2ko mice apparently respond differently to IL-1 as a function of the time after IL-1 administration. The results suggest that the early behavioral response to IL-1 is mediated primarily by the COX1 isoform, whereas COX2 appears to play a greater role in the later response.

The Effects of SC560 on mIL-1 β -Induced Changes in Milk Intake. In a series of experiments, we assessed the effects of pretreatment with the COX1-selective inhibitor, SC560 (Smith et al., 1998), on the responses to mIL-1 β at 30 and 90 min after injection. At both times, IL-1 β produced a robust reduction in milk intake (Fig. 3; $p < 0.001$). When milk intake was assessed from 30 to 40 min, SC560 at 1 and 3 mg/kg clearly attenuated the response to IL-1 (Fig. 3, top panel). There was a statistically significant interaction between the SC560 and IL-1 treatments, and the effect of IL-1 was not statistically significant in SC560-treated mice at either dose. In contrast, 90 min after injection, even though there was a statistically significant interaction between the SC560 and IL-1 treatments ($F_{7,280} = 4.26$, $p < 0.001$), the effect of IL-1 β was still statistically significant in SC560-treated mice ($p < 0.001$; Fig. 3, bottom panel). A statistically significant interaction among IL-1, drug, and time of IL-1 administration suggests that the effect of SC560 depends on the time after IL-1 administration. This supports a significant role for COX1 in early behavioral response to IL-1 β .

It has been observed in a number of experiments that administration of IL-1 β decreased the daily intake of solid food and depressed the body weight assessed 24 h later. SC560 (3 mg/kg) was administered 90 min before the onset of the dark phase and 30 min before injection of IL-1 β . The intake of food pellets and the body weight were determined at

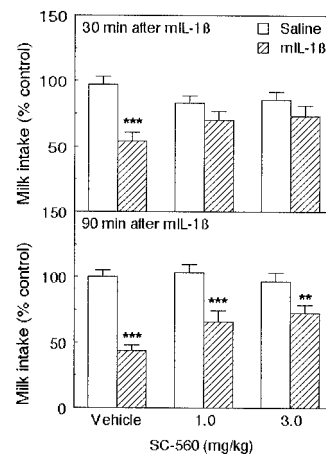


Fig. 3. The effect of the COX1-selective inhibitor, SC560, on mIL-1 β -induced reductions in milk intake in CD-1 mice. Mice were injected s.c. with SC560 (1 or 3 mg/kg) or vehicle (20% DMSO) 30 min before saline or mIL-1 β (100 ng i.p.). Sweetened milk was presented 30 (top panel) or 90 (bottom panel) min after administration of mIL-1 β . Data pooled from four independent experiments, $N = 6$ to 7 per group in each experiment. Significantly different from the vehicle-saline control group or as indicated (**, $p < 0.01$; ***, $p < 0.001$).

the beginning of the next light phase. Mice given IL-1 β displayed a smaller overnight food pellet intake (3.1 versus 4.1 g; $F_{1,33} = 28$, $p < 0.001$) and lost body weight relative to controls (-0.2 versus +0.5 g; $F_{1,33} = 22$, $p < 0.001$). No statistically significant interactions were apparent between SC560 and IL-1 ($F_{1,33} = 0.05$ and 0.160), suggesting no role for COX1 in these responses.

The Effects of Celecoxib on mIL-1 β -Induced Changes in Milk Intake. In a further series of experiments, the effects of pretreatment with the COX2-selective inhibitor, celecoxib, on the early and late IL-1-induced hypophagia were tested. A statistically significant effect of IL-1 ($F_{1,31} = 26$, $p < 0.001$; Fig. 4, left panels) was apparent 30 min after injection of mIL-1 β , but this response was not altered by even a 10 mg/kg dose of celecoxib (IL-1 \times celecoxib interaction, $p < 0.82$). This suggests that COX2 is not important for the early behavioral response. In contrast, 90 min after administration, the IL-1-induced depression of milk intake (Fig. 4, right panels; $p < 0.001$) was affected by celecoxib pretreatment (IL-1 \times celecoxib interaction, $F_{1,20} = 11$, $p < 0.001$). A dose of celecoxib as low as 1 mg/kg prevented the IL-1-induced hypophagia ($F_{1,20} = 23$, $p < 0.001$). This suggests that the response to IL-1 β 90 min after injection was mediated primarily by COX2.

The Effects of Celecoxib on LPS-Induced Reductions in Milk and Food Intake and Body Weight. Celecoxib was also tested for its ability to alter the reductions in milk intake observed 2 h after LPS administration. Figure 5 shows the results following pretreatment with 3 and 10 mg/kg celecoxib. At both doses, ANOVA revealed a significant main effect of LPS ($p < 0.01$). For 10 mg/kg celecoxib (but not 3 mg/kg), there was a significant interaction between the drug and LPS ($F_{1,66} = 23$, $p < 0.001$), indicating the attenuation by celecoxib of the LPS-induced reduction in milk intake. LPS reliably depressed overnight intake of solid food and depressed body weight ($F_{1,66} = 10.2$ and 18.8, respectively, $p < 0.01$). Celecoxib (10 mg/kg) also attenuated the effects of LPS on food pellet intake (Fig. 6; LPS \times cele-

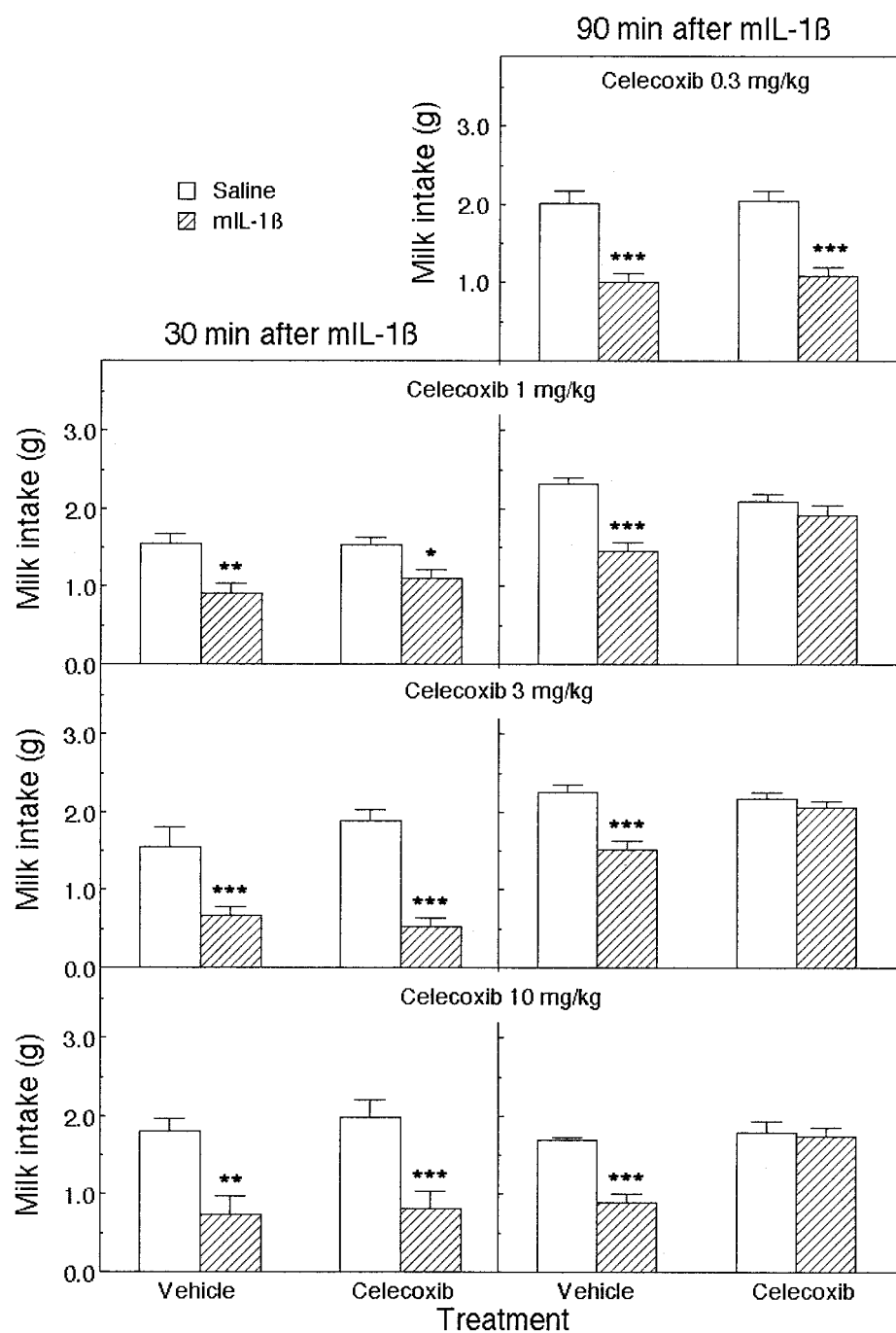


Fig. 4. The effect of the COX2-selective inhibitor, celecoxib, on mIL-1 β -induced hypophagia in CD-1 mice. Mice were injected with celecoxib (0.3, 1, 3, or 10 mg/kg) or vehicle 30 min before injection with saline or mIL-1 β (100 ng/mouse i.p.). Milk was presented 30 min after administration of saline or mIL-1 β for 10 min (left panels) or 90 min after administration of saline or mIL-1 β for 30 min (right panels). $N = 8$ to 10. Data for the 1.0 mg/kg dose were pooled from four separate experiments. Significantly different from the vehicle-saline control group (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

coxib interaction, $F_{1,66} = 3.03$, $p < 0.09$) and prevented the loss of body weight (LPS \times celecoxib, $F_{1,66} = 9.56$, $p < 0.01$).

Discussion

Interleukin-1 administration has been clearly demonstrated to decrease feeding. However, the chain of physiological events that leads from increased secretion of IL-1 to the behavioral response has not yet been established. Ample evidence indicates that COX inhibitors attenuate the effects of IL-1 (McCarthy et al., 1986; Hellerstein et al., 1989; Uehara et al., 1989; Crestani et al., 1991; Bluthé et al., 1992; Shimomura et al., 1992; Langhans et al., 1993; Swiergiel et al., 1997a; Dunn and Swiergiel, 2000). COX inhibitors are somewhat less effective in preventing the hypophagic effects

of LPS (Langhans et al., 1993; Johnson and von Borell, 1994; Swiergiel et al., 1997a). This suggests that although COX may be involved in the responses to IL-1 and LPS, other mechanisms are likely to be involved.

Cyclooxygenase occurs in two isoforms, COX1 and COX2, but which form mediates hypophagia or the other behavioral responses has not been determined. In earlier studies using selective COX antagonists, we found the COX2-selective antagonists, nimesulide and NS-398, were not particularly effective in reversing IL-1-induced reductions in milk intake, whereas the COX1-selective antagonists, piroxicam and diclofenac, were as effective as nonselective inhibitors, such as aspirin and indomethacin (Dunn and Swiergiel, 2000). However, the effect of aspirin, which is an irreversible COX in-

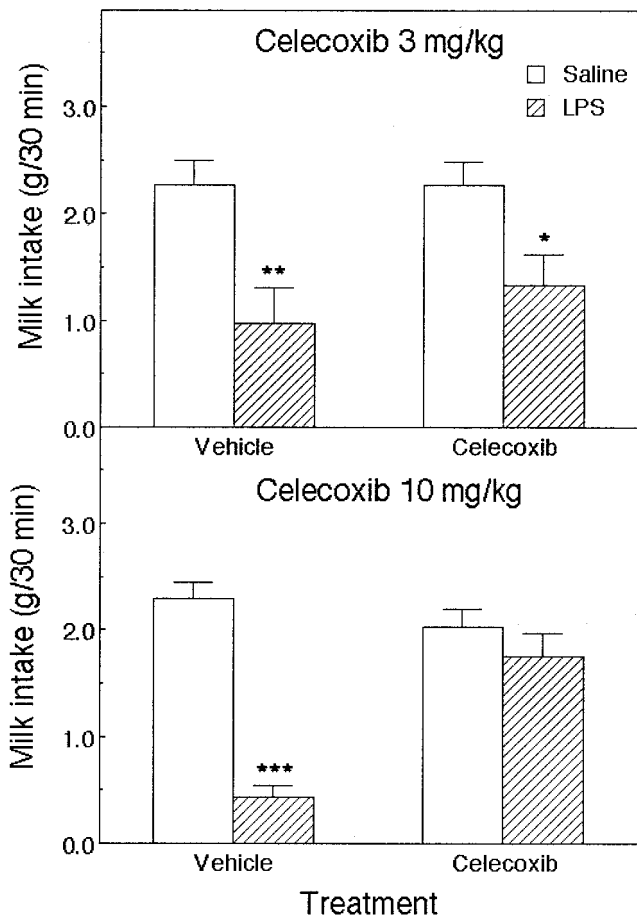


Fig. 5. Effect of celecoxib on LPS-induced hypophagia in CD-1 mice. Mice were injected with celecoxib (3 or 10 mg/kg) or vehicle followed 30 min later by saline or LPS (1 μ g/mouse i.p.). Milk was presented 120 min after administration of saline or LPS for 30 min. $N = 6$ to 10 per group in each experiment (data pooled from two separate experiments for the 10 mg/kg dose). Significantly different from the vehicle-saline control group (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

hibitor, dissipated in less than 40 h, suggesting that induced COX2 mediated the response because COX2 would be induced at this time, whereas there would be little recovery in COX1. Also, our initial studies with COX1 and COX2 knock-out mice clearly implicated COX2 in the responses to IL-1 and LPS (Swiergiel and Dunn, 2001).

The present studies reveal a more complicated picture in which the different isozymes may be involved at different times. The major finding was that animals with a genetic deficiency in COX1 (COX1ko) or treated with a selective inhibitor of COX1 (SC560) failed to display a robust response to IL-1 β at short times after its administration, suggesting that COX1 is important for this early response. Moreover, at this early time, COX2ko mice showed a normal response to IL-1 β , and the highly selective COX2 inhibitor, celecoxib, failed to alter the IL-1-induced reduction in milk intake even at a dose 10-fold higher than that which prevented the reduction in milk intake 90 min after IL-1 β .

By contrast, at the later time (90–120 min), COX1ko mice behaved like normal animals, whereas COX2ko mice showed a markedly diminished response to IL-1. Also, the response to IL-1 β was sensitive to relatively low doses of celecoxib. The response to IL-1 β was sensitive to treatment with SC560, although less sensitive than it was 30 to 40 min after IL-1 β .

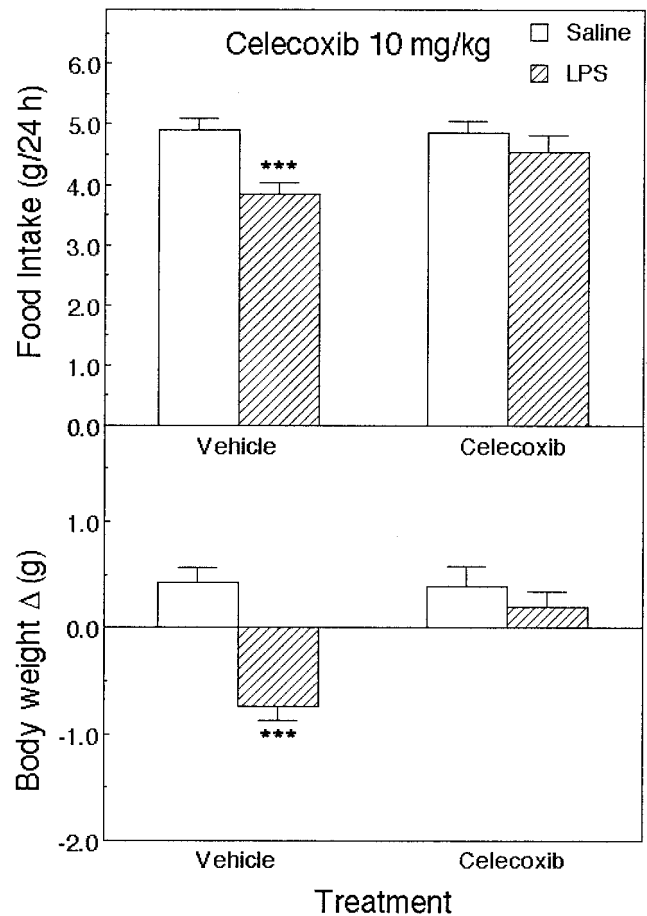


Fig. 6. Effect of celecoxib on LPS-induced changes in the overnight food pellet intake and body weight in CD-1 mice. Mice were weighed and treated with 10 mg/kg celecoxib or vehicle followed 30 min later by saline or LPS (1 μ g/mouse i.p.). Food pellets were placed in the cages 150 min after administration of LPS. The remains of the food pellets and the animals were weighed 22 h later. $N = 6$ to 10, data from the same experiments and individuals as in Fig. 5. Significantly different from the vehicle-saline control group (***, $p < 0.001$).

Thus, COX2 may be more important for the delayed response, consistent with the delayed appearance of the enzyme, which most likely first appears around 90 min after IL-1 (Cao et al., 1997; Elmquist et al., 1997; Quan et al., 1998).

Distinct physiological roles for the isozymes are also indicated by the finding that the delayed effects of IL-1 β and LPS, overnight depression of feeding and body weight, were attenuated by inhibition of COX2 with celecoxib, whereas they were not affected by inhibition of COX1 by SC560. This not only reinforces the concept that the later responses are mediated by COX2 but suggests that the COX2 response is more permanent and therefore biologically more significant. This conclusion is consistent with a recent study that suggested that COX2 was more important than COX1 in the loss of body weight and the hypophagia following LPS administration (Johnson et al., 2002).

We can only speculate on the location of the COX enzymes involved. A prime candidate is the endothelial cells in the brain. This is because when IL-1 or LPS is administered to mice and rats, COX2 is known to be induced primarily in the cerebral endothelia, whereas COX1 is unchanged (Cao et al., 1996, 1997; Quan et al., 1998). Moreover, as discussed above,

the time course of the reduction in milk intake is compatible with that of COX2 induction. Presumably, the newly induced COX2 enables the synthesis of prostaglandins that can penetrate the brain freely and activate the behavioral response. Such a mechanism would parallel that thought to be involved in the induction of fever by IL-1 and LPS, which may occur in the organum vasculosum laminae terminalis (Li et al., 1999; Blatteis et al., 2000) The brain region involved in the response in milk intake is not known, but is likely to be hypothalamic, because this structure is known to be the major one involved in the regulation of feeding. The location of the active COX1 is also unknown but could also be the cerebral endothelia, which constitutively express this enzyme (Cao et al., 1996; Quan et al., 1998).

It is not clear why COX1 is less involved at later times (the enzyme is still present), and why the milk intake response dissipates even before COX2 has reached its peak concentrations. It must be presumed that another mechanism intrudes to terminate the milk intake response to IL-1 and LPS, although the response to the latter is more prolonged (Swiergiel et al., 1997a). The mechanism for this switch and its biological significance have yet to be determined.

Most, if not all, investigations have found the feeding responses to LPS to be less sensitive to COX inhibitors than the responses to IL-1 (see above). This indicates that LPS most probably affects feeding by multiple mechanisms. It also indicates clearly that the induction of IL-1 by LPS cannot account for all the responses to LPS. This has been apparent in previous studies using IL-1 receptor antagonists, and IL-1 and IL-1 type I receptor knockout mice (Kent et al., 1992; Fantuzzi et al., 1996; Swiergiel et al., 1997b; Kozak et al., 1998; Bluthé et al., 2000; Dunn, 2000). The nature of these other mechanisms is not known, but it is relevant that there are receptors for LPS (toll-like receptor-4) on blood vessels, and that LPS administration induces profound autonomic changes and increases in plasma catecholamines (Jones and Romano, 1989).

The results of this combined genetic and pharmacological approach strongly suggest that COX1 is more important than COX2 for the early behavioral response to IL-1, although a minor role for constitutive COX2 cannot be excluded. On the other hand, the later responses seem to be much more dependent on COX2. This finding parallels the relatively slow induction of COX2, presumably as part of an inflammatory response.

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