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Pharmacology, Biochemistry and Behavior 69 (2001) 659–663

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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# Cyclooxygenase 1 is not essential for hypophagic responses to interleukin-1 and endotoxin in mice

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Received 13 March 2001; received in revised form 8 May 2001; accepted 9 May 2001

**Abstract**

Numerous studies have shown that the effects of interleukin-1 (IL-1) and endotoxin (LPS) on behavior are sensitive to cyclooxygenase (COX) inhibitors. However, neither the location of the COX involved nor the specific isoform, COX1 or COX2, is known. A previous study using selective COX1 and COX2 inhibitors did not provide an unequivocal answer. Therefore, we tested the response of sweetened milk ingestion to IL-1 and LPS in mice in which the COX1 or the COX2 gene was deleted (COX1ko and COX2ko). When IL-1β was administered 90 min before the milk, COX1ko mice showed responses similar to those of normal mice. In contrast, COX2ko mice exhibited responses considerably less than normal, with some mice showing no response. Indomethacin pretreatment almost prevented the feeding responses to IL-1 in normal and COX1ko mice. The milk intake response to LPS in COX1ko mice was like that of normal mice. The results from COX1ko mice suggest that COX1 is not necessary for the decreased milk intake following IL-1 and LPS. The results from COX2ko mice are consistent with the involvement of COX2 in the IL-1-induced depression of milk intake, but other mechanisms may effect decreases in sweetened milk intake. 

*Keywords:* Cyclooxygenase; Milk intake; LPS; Endotoxin; Interleukin-1; Knockout mice; Behavior

## 1. Introduction

Interleukin-1 (IL-1) stimulates cyclooxygenase (COX) and the production of prostaglandins (Vane et al., 1998). Numerous studies have shown that behavioral responses induced by IL-1 are inhibited by COX inhibitors (Avitsur et al., 1999; Crestani et al., 1991; Uehara et al., 1989), suggesting that COX and prostaglandins are involved in these responses. Recently, the existence of two distinct isozymes, COX1 and COX2, with different anatomical distributions and characteristics has been demonstrated (Vane et al., 1998). COX1 is constitutive, but it is distributed mainly outside the CNS. COX2 is induced as part of the inflammatory process. However, it is also constitutively expressed in certain regions of the brain, the hippocampus and cortex (Yamagata et al., 1993). It could thus play a distinct role in the behavioral responses to IL-1 or endotoxin (lipopolysaccharide, LPS). However, our previous studies with inhibitors with some selectivity towards COX1 or COX2 raised some doubts concerning the importance of COX2 in mediating the decreases in feeding in response to IL-1 or LPS (Dunn and Swiergiel, 2000). This question seems particularly appropriate for analysis with transgenic mice deficient in the COX isoenzymes. Therefore, we studied IL-1-induced behavior in mice with selective deletions of the genes for COX1 or COX2 (Langenbach et al., 1999).

## 2. Materials and methods

### 2.1. Animals

Six-week-old COX1 (B6.129P2-*Ptgs1*$^{tm1}$) and COX2 (B6.129P2-*Ptgs2*$^{tm1}$) knockout male mice were purchased from Taconic Farms (Germantown, NY) that breeds previously created COX1 and COX2 knockout mice (Langenbach et al., 1999; Morham et al., 1995). Briefly, embryonic stem cells (E14TG2a, derived from 129/OLAHsd mice)

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0091-3057/01/$ – see front matter 
PII: S0091-3057(01)00577-9

containing the appropriate targeted allele were injected into C57BL/6J (B6) blastocysts. The original chimera was mated to C57BL/6J mice. The COX1 knockout mice have been maintained on a mixed B6 × 129P2 background, whereas the COX2ko mice were maintained on a mixed 129 × C57BL/6 background. The breeder produces homozygous COX knockout mice by crossing homozygous COX knockout male with heterozygous COX knockout female and performing genotyping of the offspring. Unfortunately, we were unable to obtain mice of the appropriate background of similar age and sex when the knockout mice were purchased. Therefore, CD-1 male mice purchased from Charles River (VAF Plus Colony R16 from the Raleigh-Durham facility) were used as a control containing genes for both COX isozymes. Mice were given free access to water and Purina chow. All animals were housed at 22–23°C in individual plastic cages with wood shaving bedding under a 12 h light–dark cycle with lights on at 7 am in the AAALAC-accredited facilities. Experimental procedures have been in accordance with approved Animal Care Protocols.

### 2.2. Sweetened milk intake

Food pellet intake and the intake of sweetened condensed milk (total fat 5%; total protein 3%; total carbohydrate 7%) diluted with three parts of water were assessed as described previously (Swiergiel et al., 1997). Mice were habituated for at least 3 days to drink milk from 20 ml glass bottles fitted with metal spouts. The bottles were weighed and one placed in each cage at around 11 am. After 30 min, they were removed and reweighed to assess consumption. Animals were weighed daily and nocturnal ingestion was monitored to make sure that feeding was not disrupted and that the animals were healthy. This was done by placing two fresh and firm food pellets in the cage of each mouse overnight. The remains from the previous day were removed and weighed each morning at 8 am. Changes in body weight were followed by weighing the animals at least once a week and always before an experimental day.

### 2.3. Materials

Recombinant mouse IL-1β (mIL-1β) was purchased from R&D Systems (Minneapolis, MN) and *E. coli* LPS was from Sigma (St. Louis, MO; L3755, serotype 026:B6). mIL-1β (100 ng/mouse) and LPS (1 μg/mouse) were dissolved in sterile pyrogen-free 0.9% solution of sodium chloride such that the total dose for each mouse was contained in 0.1 ml, which was injected intraperitoneally (ip). Indomethacin (Sigma) was suspended in sterile isotonic saline with ultrasonication and injected subcutaneously (sc) at a dose of 10 mg/kg. This procedure has been shown in our laboratory to result in plasma concentrations of indomethacin adequate to inhibit COX by >90% for 16 h.

### 2.4. Experimental procedure

IL-1 was administered 90 min before and LPS 120 min before placing the milk bottles in the cage. Indomethacin was administered 30 min before IL-1. The mice were run for 11 weeks during which time they had access to sweetened milk for 30 min every weekday and on Sundays if IL-1 was administered on a Monday. Mice were rotated within the experimental groups. Each mouse received up to six injections of IL-1 but never more than once in the same week, with the single exception that in the experiment with indomethacin, a few mice received two injections in 1 week but at least 3 days apart. LPS was administered only once (during the ninth week) and to only half of the mice. There was no evidence of any change in the milk intake response to repeated IL-1 nor of any change in the response to IL-1 after LPS.

### 2.5. Motor and exploratory behavior

Mice were placed singly in the center of a multicompartment chamber (MCC). The apparatus consists of a box (38 × 38 × 23 cm) divided into nine smaller interconnecting compartments (Berridge and Dunn, 1986). Each compartment contained a 2.5 cm circular hole in the floor, beneath which a wire-mesh sphere (2.5 cm diameter) was rigidly mounted to serve as a novel stimulus for the mice to investigate. Behaviors scored included measures of locomotor and exploratory activity: the number of compartment entries and rears, the number and duration of contacts with the stimulus and the frequency and duration of grooming. Mice were observed for 25 min, 90 min after injection of saline or mIL-1β.

### 2.6. Data analysis

Most of the animals received multiple injections of mIL-1β and saline so the results obtained for each individual mouse were averaged. Two-way analysis of variance (ANOVA) was performed using SuperAnova (Abacus Concepts). The factors were genotype (CD-1 and COX1ko and/or COX2ko) and treatment (saline and IL-1 or LPS) and drug (vehicle and indomethacin). Post hoc comparisons were made using Fisher's Protected Least Significant Difference Test. All data are reported as mean ± standard error of the mean.

## 3. Results

### 3.1. General behavior

There were no differences among the three mouse genotypes (CD-1, COX1ko and COX2ko) in the locomotor activity (number of compartment entries or rears), exploratory activity (compartment entries, number of contacts

with the stimuli) or grooming. There were also no strain differences in the responses to mIL-1β; thus, these data are not presented.

### 3.2. Effects of mIL-1β on milk intake

Fig. 1 depicts the sweetened milk intake following saline or mIL-1β in CD-1, COX1ko and COX2ko mice. Two-way ANOVA performed on data collected from all three genotypes (CD-1, COX1ko and COX2ko) revealed statistically significant effects of mIL-1β ($F_{1,54}=88$, $P<.0001$), a marginal effect of genotype ($F_{2,54}=2.86$, $P<.07$) and a highly significant interaction between genotype and mIL-1β ($F_{1,54}=8.07$, $P=.001$).

To identify the source of the interaction, a two-way ANOVA was performed using data from each COX knockout genotype and the CD-1 control. ANOVA performed on the COX1ko and CD-1 mice indicated a statistically significant effect of IL-1 ($F_{1,30}=101$, $P<.0001$). There was no difference between the COX1ko and the CD-1 controls ($F_{1,30}=0.072$) and no interaction between genotype and mIL-1β ($F_{1,30}=0.236$). This indicates that the COX1ko and CD-1 mice responded very similarly to IL-1. ANOVA performed on the COX2ko and CD-1 mice indicated that administration of mIL-1β decreased milk intake ($F_{1,42}=44$, $P<.0001$), and there was a marginal effect of genotype ($F_{1,42}=3.49$, $P<.07$). Post hoc analysis showed that mIL-1β depressed drinking in both CD-1 and COX2ko mice. However, a highly significant interaction between genotype and mIL-1β ($F_{1,42}=9.72$, $P<.01$) suggests that the effects of mIL-1β were different in the COX2ko and CD-1 mice.

### 3.3. Effects of indomethacin on mIL-1β-induced changes in milk intake

In our earlier experiments, we observed that indomethacin pretreatment largely prevented the effects of IL-1β on milk intake (Swiergiel et al., 1997). Indomethacin inhibits both COX1 and COX2, although it has slightly higher potency for the former (Frölich, 1997). The effects of indomethacin pretreatment on mIL-1β-induced hypophagia were tested in both COX1 and COX2 knockouts. Mice were injected with indomethacin 30 min before mIL-1β. This pretreatment with indomethacin resulted in sickness and death in most of the COX2ko mice before any behavioral data could be collected. Fig. 2 indicates that mIL-1β reduced milk intake in the CD-1 and COX1ko mice ($F_{1,63}=8.88$, $P<.01$) as in the previous experiments. The effect of indomethacin was statistically significant ($F_{1,63}=5.40$, $P<.05$) and there was a significant interaction between indomethacin and IL-1β ($F_{2,63}=5.40$, $P<.05$), suggesting that the drug attenuated the effect of mIL-1β. In indomethacin-treated mice, the effect of IL-1 was no longer significantly different from that of the saline control. There was no significant effect of genotype ($F_{1,63}=0.35$) nor a Genotype × Indomethacin interaction, suggesting that the COX1 knockout mice responded to IL-1 and indomethacin in the same way as the CD-1 controls.

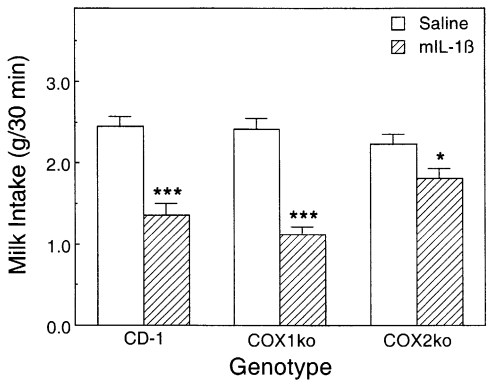


Fig. 1. Effect of administration of mIL-1β on sweetened milk intake by CD-1 and COX1 and COX2 knockout mice. Milk intake was assessed 90 min after administration of mIL-1β (100 ng/mouse ip). For CD-1, $n=10$; COX1ko, $n=7$; COX2ko, $n=13$. * Significantly different from the saline control group (* $P<.05$; *** $P<.001$).

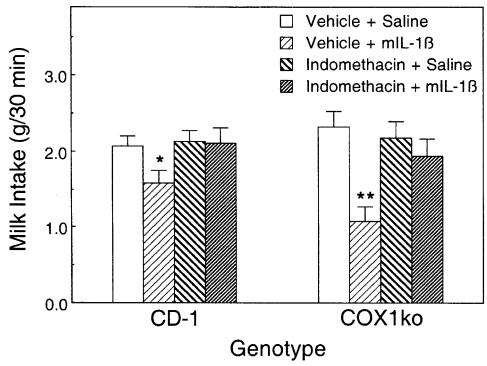


Fig. 2. Effect of indomethacin on mIL-1β-induced hypophagia in CD-1 and COX1 knockout mice. Mice were treated with indomethacin (10 mg/kg sc) followed 30 min later by mIL-1β. Milk intake was assessed 90 min after mIL-1β. For CD-1, $n=12$; for COX1ko, $n=5$. Significantly different from the vehicle + saline control group (* $P<.05$; ** $P<.01$).

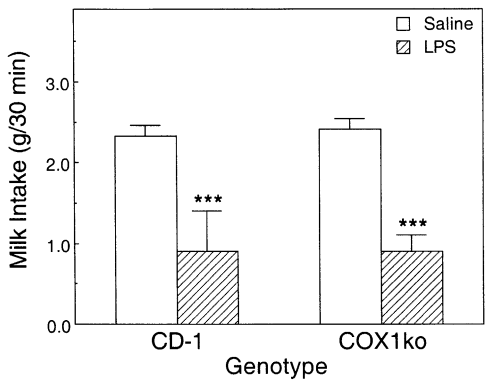


Fig. 3. Effect of LPS on sweetened milk intake in CD-1 and COX1 knockout mice. Milk intake was assessed 2 h after intraperitoneal administration of LPS (1 μg/mouse; $n=2-8$). *** Significantly different from the saline control group ($P<.001$).

### 3.4. Effects of LPS administration on milk intake

Administration of LPS produced the normal substantial reduction in milk intake in CD-1 and COX1ko mice ($F_{1,15}=48$, $P<.0001$; Fig. 3). COX2ko mice could not be tested because of the deaths caused by indomethacin. ANOVA revealed no effect of genotype ($F_{1,15}=0.038$) and no Genotype × LPS interaction ($F_{1,15}=0.038$), suggesting that the COX1ko mice responded to LPS like CD-1 controls.

## 4. Discussion

The reductions in milk intake reported here replicate the number of previous observations on the effects of IL-1 (Plata-Salamán, 1998; Swiergiel et al., 1997). However, the mechanisms of the effects of IL-1 on feeding have not been established. The issue is an important one because cytokines could be a factor mediating hypophagia in cancer and eating disorders, and as yet, there is no good pharmacological remedy.

Our previous experiments showed that the nonselective COX inhibitor, indomethacin, was effective in attenuating the effects of IL-1 on milk intake (Swiergiel et al., 1997). However, data obtained with COX inhibitors selective for COX1 and COX2 did not permit clear conclusions regarding the relative importance of either isoform (Dunn and Swiergiel, 2000). Low doses of the selective COX1 inhibitor, piroxicam, were very effective in attenuating hypophagia. In contrast, relatively high doses of the selective COX2 inhibitors, nimesulide and NS-398, were required to inhibit the responses. At those doses, it could not be excluded that COX1 was also affected. Unfortunately, our attempts to determine doses of NS-398 sufficient to block IL-1-induced fever (thought to be mediated by COX2; Li et al., 1999) were unsuccessful, because the vehicle altered core temperature. Data obtained using aspirin were more supportive of a role for COX2. Aspirin treatment impaired the milk intake response to IL-1 at 16 h, probably reflecting the irreversible inactivation of COX1 and COX2 (Wu, 1998). However, by 40 h, the sensitivity of milk intake to IL-1 had returned, possibly reflecting the induction of COX2. To resolve the issue, we tested the hypophagic responses to IL-1 in mice in which either the gene for COX1 or COX2 had been deleted.

It is important that the baseline behavior in the transgenic mice with either COX1 or COX genes deleted was very similar to that of CD-1 mice, in locomotor and exploratory activity and in sweetened milk intake. Nevertheless, the COX2ko mice were fragile and several animals died for no apparent reason and also after indomethacin treatment.

The most salient finding was that the milk intake responses to IL-1β and LPS in COX1ko mice were very much like the normal (CD-1) animals. This provides strong evidence that COX1 is not essential for these responses. The fact that indomethacin effectively prevented the milk intake response to IL-1β in COX1ko mice indicates that COX1 is not the target for this effect of indomethacin and suggests that the effect of indomethacin in wild-type mice is not mediated by inhibition of COX1. Therefore, if COX is the relevant site of action of indomethacin, COX2 is the most likely candidate. Moreover, if indeed there is no increased expression of COX2 to compensate for the absence of COX1 in COX1-deficient mice (Ballou et al., 2000), this suggests that the existing COX2 in CD-1 or COX1ko animals is sufficient to mediate a normal response to IL-1β.

A role for COX2 is also supported by the results from COX2ko mice, which showed substantially diminished responses to IL-1. It is important that the indomethacin and other COX inhibitors do not completely prevent the hypophagic responses to IL-1β (Swiergiel et al., 1997). Thus, the residual response in COX2ko mice may reflect the COX-independent response. It is relevant that the responses to LPS are even less sensitive to COX inhibitors, clearly indicating the existence of multiple mechanisms for decreasing the ingestion.

Ontogenesis in the absence of a gene can lead to compensatory changes. Thus, the abnormal responses observed in COX2ko animals may reflect a developmental change rather than the lack of COX2 per se. It has recently been shown that in mice lacking the COX2 gene, COX1 mRNA was up-regulated in the spinal cord (Ballou et al., 2000). This reinforces a previous finding that cultured lung fibroblasts obtained from COX2-deficient mice expressed greater amounts of COX1 than those from WT mice (Kirtikara et al., 1998). Nevertheless, any COX1 induction in COX2ko mice does not appear to be able to compensate for the impaired response to IL-1.

Thus, the most parsimonious conclusion of our results is that the decreased milk intake we observed in response to IL-1 depends largely on COX2. This conclusion is compatible with both the results from transgenic mice and our pharmacological studies. It is important that this conclusion can only be applied to our particular behavioral model in mice and only to administration of IL-1 and LPS injected intraperitoneally at the times we have studied it. Nevertheless, the location of the COX2 involved has not been defined. COX2 is expressed constitutively in some neurons in the hippocampus and neocortex (Yamagata et al., 1993). However, COX2 is also induced in brain endothelial cells following IL-1 and LPS administration (Cao et al., 1995, 1996). The time course of these responses in rats indicates that COX2 mRNA appears around 1 h after IL-1 or LPS and protein significantly later. This is partially, but not completely, compatible with the time course of the milk-drinking responses to IL-1 and LPS in mice (Swiergiel et al., 1997). Thus, induction of COX2 in brain endothelia may represent a mechanism for the responses we observe to IL-1 and LPS, but it may not be the only one.

In summary, the present results suggest that COX1 is not required for the decreases in sweetened milk intake we observe in mice after intraperitoneal injection of IL-1 and

LPS. These and the existing pharmacological data suggest that COX2 is the main isozymes involved under our experimental conditions.

## Acknowledgments

This research was supported by a grant from the National Institute of Neurological Diseases and Stroke (NS35370). The technical assistance of Mr. Glen Farrar and Mrs. Galina Mikhaylova is greatly appreciated.

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