The viral mimic, polyinosinic:polycytidylic acid, induces fever in rats via an interleukin-1-dependent mechanism

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Fortier, Marie-Eve, Stephen Kent, Helen Ashdown, Stephen Poole, Patricia Bokska, and Giamal N. Luheshi. The viral mimic, polyinosinic:polycytidylic acid, induces fever in rats via an interleukin-1-dependent mechanism. Am J Physiol Regul Integr Comp Physiol 287: R759–R766, 2004.—Polyinosinic:polycytidylic acid (poly I:C) is a synthetic double-stranded RNA that is used experimentally to model viral infections in vivo. Previous studies investigating the inflammatory properties of this agent in rodents demonstrated that it is a potent pyrogen. However, the mechanisms underlying this response have not been fully elucidated. In the current study, we examined the effects of peripheral administration of poly I:C on body temperature and cytokine production. Male rats were implanted with biotelemetry devices and randomly assigned to one of the following three groups: poly I:C + saline, poly I:C + interleukin-1 receptor antagonist (IL-1ra), or saline + saline. Maximal fever of 1.6°C above baseline was observed 3 h after an intraperitoneal injection of poly I:C (750 µg/kg). Pretreatment with IL-1ra diminished this response by >50% (maximum body temperature = 0.6°C above baseline). Plasma IL-6 concentration increased fivefold 2 h post-poly I:C compared with saline-injected rats; levels returned to baseline 4 h postinjection. Pretreatment with IL-1ra prevented this rise in IL-6. Plasma tumor necrosis factor (TNF-α) was also increased more than fourfold 2 h postinjection but remained unaffected by IL-1ra treatment. IL-1β and cyclooxygenase-2 mRNA were significantly upregulated in the hypothalamus of poly I:C-treated animals. Finally, poly I:C decreased food intake by 30%, but this response was not altered by pretreatment with IL-1ra. These results suggest that poly I:C induces fever, but not anorexia, through an IL-1 and prostaglandin-dependent mechanism.

cytokines; fever; food intake

AMMUNITION OF BACTERIAL endotoxin [lipopolysaccharide (LPS)] to laboratory animals is a widely used model of infection and inflammation. Similar to infection with live pathogens, LPS induces fever and a variety of sickness behaviors, including decreased food intake, weight loss, and increased sleep. The mechanisms underlying LPS-induced fever and sickness behaviors have been well characterized and shown to be mediated by proinflammatory cytokines, the most important being interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF-α).

Unlike bacterial infection, little is known about sickness responses to viral infection. Because most viruses produce double-stranded RNA (dsRNA) at some point during their replication (17), a synthetic viral-like dsRNA that stimulates antiviral activities of the innate immune system, polyinosinic: polycytidylic acid (poly I:C), has been used to mimic viral infections. Advantages of using poly I:C over live viruses include safety, convenience, but more importantly reproducibility and control over dose and time of administration of the immunological challenge. Similar to LPS, central or systemic administration of poly I:C results in the induction of the acute-phase reaction, fever, and sickness behaviors in a variety of species, including mice, rabbits, guinea pigs, and rhesus monkeys (11, 16, 19, 20, 48). Most studies examining the pyrogenic properties of poly I:C have been carried out in rabbits injected intravenously with low doses (2.5–50 µg/kg) of poly I:C, which resulted in fevers of ∼1°C in magnitude (20, 22, 46). Studies using rats or mice reported comparable increases in body temperature in response to higher doses of poly I:C (20–600 µg/animal; see Refs. 10, 15, 32, 48). However, none of these studies examined the poly I:C-induced febrile response in relation to cytokine expression. Thus, in contrast with infection models using LPS where the cytokine cascade leading to fever and sickness behavior has been extensively studied (13, 29), the mechanisms underlying the pyrogenic response to poly I:C have not been fully elucidated.

Poly I:C is best known as a potent inducer of interferon (IFN)-α and -β in vitro and in vivo (32, 34). It has also been reported to induce IL-6, IL-12, and TNF-α in vitro in human and mouse leukocyte cultures (2, 34). Proinflammatory cytokine mRNAs were also detected in the lungs of mice inoculated intratracheally with poly I:C (49). In contrast, very little is known about the poly I:C-induced plasma profiles of the three principal proinflammatory cytokines (IL-1β, IL-6, and TNF-α), and no studies have explored the role of these cytokines in the pyrogenic response to poly I:C treatment. Thus the aim of the present study was to examine the effects of peripheral administration of poly I:C on body temperature, food intake, and cytokine production and to test if the febrile effects of poly I:C, like those induced by LPS, are IL-1 dependent.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats (250–300 g body wt, Charles River, Quebec, Canada) were used in all experiments. The animals were housed individually in a controlled environment at an ambient temperature of 21 ± 2°C and a 12:12-h light-dark cycle (lights on from 0800 to 2000). Food and water were provided ad libitum. In one study, powdered food (powdered 5012; Ralston Purina, Ottawa, Can-

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RT-PCR. To investigate brain mechanisms involved in poly I:C-induced fever, we examined two markers of inflammation, namely IL-1β and cyclooxygenase (COX)-2. We measured mRNA rather than protein since both markers occur in relatively low levels in the hypothalamus after systemic injections of inflammatory stimuli. Two hours after injection with either saline (n = 5) or poly I:C (750 μg/kg ip, n = 6), animals were perfused intracardially with diethyl pyrocarbonate saline. Brains were removed, frozen on dry ice, and stored at –80°C. The hypothalami were then dissected before use and homogenized in TRizol Reagent (Invitrogen), and total RNA was isolated following the manufacturer’s instructions. The air-dried RNA pellet was dissolved in 100 μl autoclaved water, and cDNA was synthesized using murine myeloleukemia virus RT (MMLV; Invitrogen). RNA (1 μg) was incubated with random primers (5 μM; Applied Biosystems) for 10 min at 65°C, and cDNA synthesis was performed by adding dithiothreitol (10 μM; Invitrogen), MMLV RT (200 units), dNTPs (1 mM; Sigma-Aldrich), and first-strand buffer (Invitrogen) and incubating for 1 h at 37°C, followed by 5 min at 90°C to inactivate the enzyme. PCR amplification of cDNA was performed using ReadyMix RedTag PCR Reaction Mix with 1.5 mM MgCl2 (Sigma-Aldrich) and 6 pmol primers for β-actin (forward: 5'-GCGCTTCTTCCCCATCTGCTGTG-3'; reverse: 5'-TAGACCGACGGTCATACAGGGAAGACTACT-3'), IL-1β (forward: 5'-CCCGCCAGCGCTTTCTCTTCTCATCTT-3'; reverse: 5'-CAGGGTGTTGTGCGCTTTTCTT-3'), or COX-2 (forward: 5'-GCTTACAGAAGCGATGCAAA-3'; reverse: 5'-TGATGGTCTGCTGGTGGAA-3'), using a Gene Amp PCR system 9700 Thermocycler (Applied Biosystems). The linear phase of PCR amplification was determined by performing RT-PCR on a sample from each treatment group for an increasing number of cycles (20–50). The optical density (absorbance) of each PCR product was quantified by densitometry using Northern Eclipse version 6.0 (Empix Imaging) and plotted against the number of cycles. A cycle number within the exponential phase of the reaction was then selected and used for all subsequent PCRs. The following cycling parameters were used: 1) 5 min at 94°C; 2) 30 s at 94°C, 30 s at 60°C, followed by 45 s at 72°C (β-actin and IL-1β) or 2 min at 72°C (COX-2), for 26, 32, and 46 cycles, respectively; and 3) 72°C for 10 min. The absorbance of the PCR products was quantified and expressed as a percentage of actin absorbance [relative absorbance (gene X/actin mRNA × 100)].

Statistical analysis. Temperature measurements were averaged over 30 min and then analyzed using two-way ANOVA, with treatment as the between-subjects factor and time as a within-subjects repeated measure. Significant interactions between time and treatment were decomposed using simple main effects tests, and post hoc pairwise comparisons were performed to assess differences between specific treatments at a given time point. Food intake and body weight data were analyzed in the same fashion. ELISA results were analyzed with one-way ANOVA and RT-PCR data with Student’s t-tests. Post hoc Newman-Keuls’ tests were performed where indicated. Statistical analyses resulting in probabilities of P < 0.05 were considered significant.

RESULTS

Poly I:C induced fever and decrease in food intake. Intraperitoneal injection of 750 or 1,000 μg/kg poly I:C induced a significant rise in body temperature compared with saline-treated controls, starting at 120 and 150 min after injection, respectively, and remaining significant up to 420 min (Fig. 1; all P < 0.05). The fever appeared monophasic in both cases, although all groups of animals exhibited a transient hyperthermia at 30 min, most likely because of handling stress during the injection. Interestingly, the largest increases in body temperature were observed after the administration of the medial dose of poly I:C (750 μg/kg), with a maximal amplitude of...
1.6°C at 3 h. The largest dose of poly I:C, 1,000 µg/kg, induced a rise in body temperature peaking 4.5 h after injection at 1°C above baseline. The difference between these two doses was significant early in the course of the febrile response [P < 0.05 at time (t) = 120 and 180 min]. The lowest dose of poly I:C (500 µg/kg) did not significantly alter body temperature compared with the saline-treated controls, except for two time points (t = 240 and 300 min).

In a second experiment, injection of poly I:C (750 µg/kg) again resulted in a significant fever 120–420 min after injection (Fig. 2, all P < 0.01), which reached a peak amplitude of 1.1°C at t = 150 min. Administration of IL-1ra at 0 and 1 h after poly I:C injection significantly attenuated the febrile response (Fig. 2, P < 0.05 at t = 120–330 min for poly I:C + saline vs. poly I:C + IL-1ra). However, the poly I:C + IL-1ra-treated group still displayed a significantly higher body temperature than the saline controls (P < 0.05 at t = 210–330 and 390 min), with a peak difference of 0.6°C at t = 240 min. The fevers lasted for 7 h (Fig. 2), after which body temperature returned to baseline (8–48 h, data not shown).

In the same experiment, poly I:C alone reduced food intake by 31% over the 24-h period after treatment (Fig. 3A, P < 0.01), with the animals eating, on average, 8.3 g less food than was consumed over the same period before treatment (Fig. 3A, P < 0.01). Poly I:C-treated animals lost an average of 3.3 ± 1.9 g body wt in the 24 h after treatment, whereas their controls gained an average of 5.0 ± 1.0 g/day in each of the 2 days after saline injection (Fig. 3B, P < 0.05). These anorectic effects of poly I:C lasted for 24 h, after which food intake returned to baseline levels. To study the role of IL-1 in poly I:C-induced anorexia, a third group of rats was injected one time with poly I:C (750 µg/kg) and with IL-1ra (1 mg/kg) at 0 and again at 1 h. In contrast to its effects on body temperature, IL-1ra had no effect on the decrease in food intake or weight loss induced by poly I:C. Animals treated with poly I:C + IL-1ra consumed...
significantly less food (8.8 g or 33%) over the 24-h period after the injection compared with the same period before treatment (Fig. 3A, \( P < 0.01 \)). They also exhibited a 5.2 \( \pm \) 3.5 g decrease in body weight in the 24 h after treatment (Fig. 3B, \( P < 0.01 \)), which was similar to the poly I:C alone-treated group. All groups of animals returned to baseline food consumption levels 48 h after treatment (day 2; Fig. 3A). Poly I:C alone and poly I:C + IL-1ra animals gained more weight 48 h after treatment (day 2) than on the day preceding the treatment (day 0; \( P < 0.05 \)). The saline group did not show this rebound effect.

**Inflammatory cytokine response to poly I:C.** The decrease in fever resulting from the concurrent administration of IL-1ra and poly I:C suggests that IL-1 plays an integral role in the pyrogenic response to poly I:C. To further investigate the role of this cytokine, as well as that of IL-6 and TNF-\( \alpha \), we measured the plasma concentration of these mediators in a separate study. These experiments revealed that plasma IL-1\( \beta \) concentrations were close to the detection limit of the assay and did not deviate significantly from baseline levels (\( t = 0 \)) at any of the time points tested (Fig. 4A). In contrast, poly I:C administration resulted in a fivefold increase in plasma IL-6 concentrations, which peaked at 358 \( \pm \) 43 pg/ml 2 h after the injection (Fig. 4B, \( P < 0.01 \) compared with baseline). The increase in plasma IL-6 was transient, with levels returning to baseline 4 h after the injection, where they remained until the end of the study (8 h). A similar trend was seen with TNF-\( \alpha \), where a fourfold elevation in plasma TNF-\( \alpha \) was observed 2 h postinjection (267 \( \pm \) 60 pg/ml; Fig. 4C, \( P < 0.01 \)), whereas no significant changes from baseline levels were observed at the other time points tested.

To determine whether the poly I:C-induced increase in plasma IL-6 was IL-1 dependent, poly I:C-injected rats were pretreated with IL-1ra in a separate experiment. After the poly I:C injection (2 h), poly I:C alone induced a significant rise in plasma IL-6 (134 \( \pm \) 48 pg/ml; Fig. 5, \( P < 0.05 \)) compared with saline or IL-1ra controls, although values for the latter two groups did not deviate from baseline. The poly I:C-induced rise in plasma IL-6 concentration was attenuated significantly in the presence of IL-1ra (value for poly I:C + IL-1ra = 49 \( \pm \) 19 pg/ml). In the same study, the poly I:C-induced increase in plasma TNF-\( \alpha \) concentration was not affected by IL-1ra treatment (data not shown).

In a separate study designed to examine the brain mechanisms underlying poly I:C-induced fever, the hypothalami of saline- and poly I:C-treated rats were microdissected, and COX-2 and IL-1\( \beta \) mRNA, two well-established inflammatory mediators in the central nervous system, were analyzed using semiquantitative PCR. Poly I:C induced an approximately twofold increase in IL-1\( \beta \) mRNA compared with saline-treated controls (Fig. 6A, \( P < 0.01 \)). Similarly, hypothalamic COX-2 mRNA, which appears downstream of IL-1\( \beta \) during fever and is an important indicator of pyrogenic activity, was significantly higher in poly I:C-treated animals relative to saline controls (Fig. 6B, \( P < 0.01 \)). Analysis of the linear relationship between IL-1\( \beta \) and COX-2 mRNA in the poly I:C- and saline-treated animals revealed that the slopes and intercepts of both regression lines were not significantly different, making the analyses of the linear regression of the combined data possible. Analysis of pooled data from poly I:C- and saline-treated animals revealed a significant positive correlation between hypothalamic IL-1\( \beta \) and COX-2 mRNA levels (Fig. 6C;
In the present study, we demonstrate that poly I:C induces a significant long-lasting fever in rats that is comparable in magnitude and time course to that observed after treatment with LPS. A dose-response study using this synthetic viral product showed that the animals responded maximally to a dose of 750 μg/kg ip (Fig. 1). Although the doses in our study are relatively high compared with those used in studies with rabbits [e.g., 5 and 33 μg/kg iv (22)], they are consistent with those used in other rodent species [e.g., guinea pig: 800 μg/kg im (11); mouse: 600 μg/animal ip (48)]. Whereas LPS-induced fever in rats is generally biphasic (42), the febrile response to poly I:C in the present study was monophasic, starting at 2 h, peaking at 3 h, and returning to basal temperatures at 8 h. Interestingly, the highest poly I:C dose induced a fever response that was significantly lower than that resulting from the medial dose. This suggests that the dose response has a “bell-shaped” pattern, an observation made previously by others in mice injected with poly I:C (49).

The most significant observation in this study is that IL-1ra significantly attenuated the febrile response to poly I:C, thus demonstrating for the first time that the pyrogenic action of poly I:C is IL-1 dependent. This is similar to our previous findings and those of others using LPS in the presence and absence of IL-1ra (27, 45), suggesting a common axis between the inflammatory responses caused by the two stimuli. Curiously, we failed to detect any changes in the levels of circulating IL-1β at any time point examined; however, this was also observed in our earlier studies using LPS (27). We have previously shown that the contribution of endogenous IL-1β to the febrile response to exogenous inflammatory stimuli is most likely at the level of the local site of inflammation (9, 35). Locally induced IL-1β will in turn result in the induction of circulating IL-6, which acts as a pyrogenic signal to the brain (9, 35). This hypothesis is consistent with the results of the present study, since the poly I:C-induced rise in plasma IL-6 was inhibited by IL-1ra. However, in contrast to LPS, which resulted in a significant increase in this cytokine over an extended period (1–6 h after LPS; see Ref. 27), the effect of poly I:C was relatively transient. A significant increase in circulating IL-6 levels was only detected 2 h after the injection, during the rising phase of the fever. This suggests that IL-6 may only act as a trigger for poly I:C-induced fever and that other pathway(s) are involved in maintaining the pyrogenic response. Although the absolute plasma IL-6 concentrations 2 h after treatment with poly I:C were different between the time course study (358 pg/ml; Fig. 4B) and that involving IL-1ra pretreatment (134 pg/ml; Fig. 5), analysis of the data indicated that the quality controls performed on each ELISA plate used in our experiments fell within a 10% range of the expected values. Furthermore, an interassay coefficient of variation of 1.05% indicated that our assays are reliable. Variability in plasma IL-6 observed between the two experiments may be because of the fact that the two studies were performed at different times, using different animals. This could have been a significant factor since subtle variations in the way animals are raised can affect several parameters in the adult animal, including their response to stressors (3). Regardless of these differences, it is important to note that, in both experiments, the increase in IL-6 concentrations relative to control (i.e., 5-fold) was the same.

In contrast to IL-6, IL-1ra failed to attenuate the poly I:C-induced increases in plasma TNF-α concentrations, suggesting that the upregulation of this cytokine in the circulation is not IL-1 dependent. A difference was also observed between the time course of the plasma TNF-α response to poly I:C and to LPS from earlier studies. Similar to IL-6, significant increases in circulating TNF-α were only observed 2 h after the poly I:C injection. In contrast, TNF-α is the first cytokine normally detected at the site of inflammation and in the plasma after treatment with LPS, where it reaches maximal levels as early as 1 h after the injection (26), with IL-6 generally peaking 2–4 h after treatment (26, 28). This suggests that poly I:C might trigger a different peripheral cytokine cascade than the one induced by LPS. The difference in the time profiles between IL-6 and TNF-α does not preclude the latter from involvement in mediating the fever response to poly I:C, especially since IL-1ra failed to completely abolish the increase in body temperature. We have previously demonstrated that TNF-α is involved in mediating fever after localized inflammation in rats (31), and others have shown that it induces COX-2 in the brain (8). The role of TNF in fever, however, remains controversial, with some suggesting that it acts as an endogenous cryogen (26). The involvement of this cytokine in poly I:C-induced fever will need to be explored further in future studies.

At the level of the brain, our results suggest that poly I:C induces fever through similar central mechanisms to those triggered by LPS. Similar to LPS-induced fever (5, 37), hypothalamic IL-1β and COX-2 mRNA were both significantly upregulated by poly I:C in the present study. COX-2, an enzyme responsible for prostaglandin synthesis, is essential for the generation of the febrile response to LPS or pyrogenic cytokines such as IL-1 (7, 25). COX activity appears to be equally important for the mediation of poly I:C-induced fever, where inhibition of this enzyme was demonstrated to signifi-

Fig. 5. Plasma IL-6 induction by poly I:C in the presence or absence of IL-1ra. IL-1ra treatment (1 mg/kg, −1 and 0 h) significantly attenuated the poly I:C (750 μg/kg ip)-induced rise in plasma IL-6 (poly I:C + saline and poly I:C + IL-1ra, n = 6; IL-1ra + saline and saline + saline, n = 5). P < 0.05, poly I:C + IL-1ra vs. poly I:C (#) and poly I:C + saline vs. IL-1ra + saline and saline + saline (*).
cantly attenuate the febrile response in rabbits (1, 43, 44). In the current study, the linear relationship between levels of IL-1β and COX-2 mRNA after poly I:C treatment also suggests that COX-2 is induced by IL-1β in the course of poly I:C-induced fever, since it is in the course of fever induced by other inflammatory agents, such as LPS (6, 24). These observations suggest that the mechanisms involved in triggering the febrile response to poly I:C include the induction of all three proinflammatory cytokines tested, with IL-6 probably acting as the circulating signal to the brain. In addition, we provide some evidence to suggest that, at the level of the hypothalamus, both IL-1β and subsequently COX-2 are involved in activating the febrile response to systemic viral stimulation.

In contrast to poly I:C-induced fever, which was largely mediated by IL-1, decreases in body weight and food intake appeared totally insensitive to the administration of IL-1ra. This contrasts with LPS-induced anorexia, which is inhibited by IL-1ra (14, 23). It is unlikely that the lack of effect of IL-1ra on the poly I:C-induced reduction in food intake is because of loss of activity over time, since we have previously demonstrated that a similar dose of IL-1ra inhibited the effect of the appetite suppressant leptin over a 22-h period (30). The observations made in the present study exclude IL-1 and, by extension, IL-6 as mediators for poly I:C-induced anorexia but do not, however, exclude TNF-α, which increases significantly after poly I:C treatment and is not inhibited by IL-1ra. This cytokine has previously been implicated in mediating a decrease in food intake after infection and inflammation (38, 47) and provides a viable alternative to IL-1 and IL-6 for mediating the anorexic effects of poly I:C. However, this would need to be confirmed by neutralization studies in vivo. Other cytokines may also be involved. For example, IFN-α, which is produced in significantly high concentrations in response to poly I:C (39), has been shown to decrease food intake and activity in humans and mice (12, 40), and is suggested to play a role in the poly I:C-induced decrease in physical activity (18).

In addition to humoral signals, other pathways have been described that can relay the peripheral inflammatory signal to the brain, namely afferent fibers of the vagus nerve. Vagal afferents have been shown to mediate some of the LPS-induced behavioral effects (e.g., decreased social interaction, decreased food intake, and reduction in food-motivated behavior; see Refs. 4, 21, and 33). It is therefore possible that vagal afferents play a role in poly I:C-induced anorexia, using central IL-1-independent mechanisms, although no direct evidence exists to implicate this route of propagating the peripheral poly I:C-induced inflammatory signal to the brain.

In conclusion, the current study clearly demonstrates that effects of poly I:C on fever and on food intake are mediated via differing pathways, with effects on fever being IL-1 dependent and those on food intake IL-1 independent. Further studies are needed to explore these pathways in greater detail.

Fig. 6. Effect of poly I:C on brain IL-1β and cyclooxygenase (COX)-2 mRNA. IL-1β (A) and COX-2 (B) mRNA were upregulated significantly in the rat hypothalamus in response to peripheral poly I:C injection (750 μg/kg ip, n = 6) compared with saline treatment (n = 5). **P < 0.01. C: in addition, analysis of combined data from saline- and poly I:C-treated animals revealed that hypothalamic levels of mRNA for IL-1β and for COX-2 were significantly correlated (r = 0.88, P < 0.01).
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