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The role of prostaglandins in the nociceptive response induced by intraperitoneal injection of zymosan in mice

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1 Intraperitoneal injection of zymosan (1 mg in 0.5 ml saline) in mice induces a transient writhing response accompanied by the synthesis of small amounts of prostaglandin E_2 (PGE_2, < 2 ng) and larger amounts of PGI_2 (200 ng per mouse), measured as its non-enzymatic breakdown product, 6-keto-$PGF_{1\alpha}$.

2 Although both centrally-acting analgesics (morphine, clonidine) and prostaglandin biosynthesis inhibitors (aspirin, indomethacin, ibuprofen) blocked the writhing response to intraperitoneal injection of zymosan, only the latter reduced prostaglandin levels in the peritoneal cavity.

3 The writhing response correlated equally well with PGE_2 levels and 6-keto-$PGF_{1\alpha}$ levels when data from mice treated with centrally-acting analgesics were excluded. However, intraperitoneal injection of PGI_2, but not PGE_2, reversed the analgesia induced by indomethacin in zymosan-injected mice.

4 Centrally-acting agents, but not ibuprofen, blocked the ability of PGI_2 to reverse the analgesic activity of indomethacin.

5 PGI_2 (2 μg per mouse), injected intraperitoneally in otherwise untreated mice, induced writhing.

6 These data indicate that PGI_2 is the prostaglandin involved in mediation of the writhing response to zymosan and that prostaglandin biosynthesis inhibitors, but not centrally-acting analgesics, exert their analgesic activity by reducing the peritoneal level of PGI_2. It is possible that PGI_2 may have the ability to stimulate pain receptors directly in the mouse peritoneal cavity, in addition to its previously recognized ability to sensitize pain receptors to other pain-producing stimuli.

Introduction

It has been clearly shown that prostaglandin E_1, E_2 and I_2 (PGE_1, PGE_2, PGI_2) produce hyperalgesia (Ferreira, 1972; Ferreira *et al.*, 1973; Ferreira *et al.*, 1978b; James & Church, 1978). This phenomenon has been used to explain both the hyperalgesia which occurs in inflamed tissues in which prostaglandin levels are elevated, and the analgesic effects of prostaglandin biosynthesis inhibitors in such situations (Vane, 1971; Moncada *et al.*, 1978; Ferreira, 1981). However, it is not yet clear which of the prostaglandins found in inflamed tissues plays the most important role in the hyperalgesia of inflammation. Also, the quantitative relationships between prostaglandin levels and the pain response have not been delineated. A recently described model, the nociceptive response and inflammation induced by intraperitoneal injection of zymosan in mice (Doherty *et al.*, 1984; Doherty *et al.*, 1985a,b), offers the opportunity to resolve some of these questions. In this model, it has proved possible to measure the levels of several relevant arachidonic acid metabolites, in addition to quantifying the inflammatory and nociceptive (writhing) responses. The present report describes studies with this model which identify PGI_2 as the prostaglandin of greatest importance in the writhing response to zymosan and clarify the mechanism of action of some analgesic agents. A preliminary report of this work was presented at the 2nd World Congress on Inflammation, Antirheumatics, Analgesics, Immunomodulators (Monaco, March 19–22, 1986).

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Methods

The methods for the study of zymosan-induced writhing and inflammation have been described previously (Doherty *et al.*, 1985a). Briefly, this involves intraperitoneal injection of 1 mg of zymosan (Sigma

Chemical Co, St. Louis, MO, U.S.A.), suspended in 0.9% w/v sodium chloride (0.5 ml, unless otherwise indicated) into male mice (CD1, Charles River Breeding Labs, Wilmington, MA, U.S.A., 20–35 g body weight) which had been pretreated with test compounds or vehicle. Writhes were counted by an observer unaware of the allocation of treatments. At an appropriate time after injection of zymosan, the mice were killed by cervical dislocation and the peritoneal cavity lavaged with 2 ml of saline. Proteins were removed from the lavage fluid by addition of ethanol to 80% v/v followed by centrifugation. The supernatants were dried at 40°C under a constant stream of nitrogen and stored at −70°C until assayed.

For assays by gas chromatography/mass spectrometry (g.c./m.s.), prostaglandins were first extracted (Powell, 1980) and then derivatized (Waddell *et al.*, 1983) as follows: To each tube of dried peritoneal fluid was added deuterated $PGF_{2\alpha}$, as internal standard, and 1 ml of water adjusted to pH 3 with 1N HCl. After vigorous vortexing, the sample was passed over a preconditioned column of octadecylsilyl silica (C18 minicolumn, J.T. Baker, Phillipsburg, NJ, U.S.A.), which was washed with 2 ml aliquots of water, ethanol/water (15:85) and petroleum ether, then eluted with 2 ml of ethyl formate. The ethyl formate was removed by evaporation under nitrogen and carbonyl groups derivitized with 100 µl of 5 mg·ml^{-1} methoxyamine hydrochloride in pyridine (38°C for 1 h). After drying under nitrogen, the carboxylic acid groups were derivitized with 30 µl of acetonitrile, 10 µl of pentafluorobenzylbromide (35%) in acetonitrile and 10 µl diisopropylethylamine at 40°C for 15 min. This derivative permits the use of negative chemical ionization in the electron capture mode as a sensitive and selective detection system. Remaining hydroxyl groups were derivatized, after drying under nitrogen, with 100 µl of *bis*-trimethylsilyl-trifluoroacetamide, overnight at room temperature. After drying under nitrogen and dissolving in 200 µl of hexane, 1 µl was injected into a 15-meter narrow bore (0.25 µm diameter) capillary column (DB5, J&W, Rancho Cordova, CA, U.S.A.). A Finnigan MAT g.c/ms. (Model 4600) (Finnigan MAT, San Jose, CA, U.S.A.) operated in the negative ion chemical ionization mode with methane as the reagent gas was used. The column eluate was monitored by selected ion monitoring at M/Z 573 (deuterated $PGF_{2\alpha}$), 524 (PGE_2, PGD_2) and 614 (6-keto-$PGF_{1\alpha}$, thromboxane B_2 (TxB_2). Elution times of standards are shown in Figure 3.

For radioimmunoassay (RIA) analysis of PGE_2 and 6-keto-$PGF_{1\alpha}$, the dried peritoneal fluids were reconstituted with 1 ml of water and centrifuged at 10000 *g* for 10 min. The supernatants were diluted with water and the assay performed as described by the manufacturer of the I^{125} RIA kits (New England Nuclear, Boston, MA, U.S.A.).

TxB_2, 6-keto-$PGF_{2\alpha}$, PGE_2, $PGF_{1\alpha}$ and PGI_2 (sodium salt) were obtained from Upjohn Diagnostics (Kalamazoo, MI, U.S.A.) or Chemical Dynamics Corp. (South Plainfield, NJ, U.S.A.) and were stored at −70°C. Tetradeuterated $PGF_{2\alpha}$ was obtained from MSD Isotopes (Montreal, Canada). For *in vivo* studies, PGE_2 was stored as an ethanolic solution and, just before use, the ethanol was evaporated under a stream of nitrogen at room temperature. The PGE_2 was then dissolved in pH 10 carbonate buffer. PGI_2 was dissolved in the same ice-cold buffer just before injection. The use of this buffer was necessary to minimize the spontaneous breakdown of PGI_2 before administration (Cho & Allen, 1978).

For oral administration, compounds were prepared as suspensions in water containing 2 drops of Tween 80 (Fisher Scientific, Fairlawn, NJ, U.S.A.) per 10 ml and were homogenized in a teflon-in-glass homogenizer to reduce particle size. The dose volume used was 10 ml kg^{-1}. For subcutaneous administration, compounds were prepared in saline. The drugs used were obtained from the following sources: aspirin and indomethacin (Sigma Chemical Co.), ibuprofen (Upjohn, Kalamazoo, MI, U.S.A.), morphine (Merck & Co., Daarmstaad, F.R.G.), clonidine (Boehringer Ingelheim, Ridgefield, CT, U.S.A.), pentazocine (Sterling-Winthrop, Rensselaer, NY, U.S.A.). Animals were allocated to treatment randomly. Treatment groups were compared with controls by analysis of variance followed by Dunnett's test (Steel & Torrie, 1960), or, when homogeneity of variance problems made analysis of variance inappropriate by multiple *t*-tests, with Bonferoni's adjustment (Neter & Wasserman, 1974). For comparison of proportions (e.g. % of mice writhing), Fisher's exact test was used and for assessment of the significance of the linear relationship between parameters, least squares regression was performed (Steel & Torrie, 1960).

Results

It was previously reported (Doherty *et al.*, 1985a) that 1 mg of zymosan (in an injection volume of 0.5 ml) produced the optimum writhing response; higher doses administered in the same volume did not increase the number of writhes. As shown in Figure 1, when 1 mg was administered in 5 ml of saline, the response was much reduced and occurred with a longer latency after injection. When 1 mg was administered in 0.05 ml of saline, the response was similar in time course to that of 1 mg per 0.5 ml, but much reduced in magnitude (Figure 1). Therefore, in all subsequent studies, 1 mg per 0.5 ml has been used and writhes counted for a 15 min period, either 5–20, 10–25 or 15–30 min after injection of zymosan. The 15 min period selected for a particular experiment was

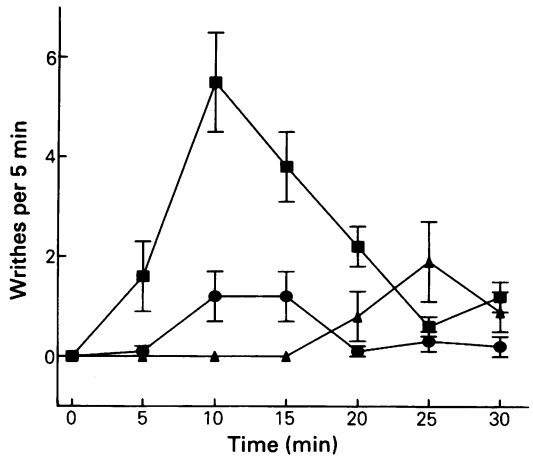


Figure 1 The effect of dose volume on the writhing response to zymosan Mice were injected i.p. with 1 mg of zymosan suspended in 0.05 (●), 0.5 (■) or 5 (▲) ml of 0.9% w/v saline. Writhes were counted over 5 min intervals for each mouse. The points represent the mean ($n = 14-20$) for the preceding 5 min; vertical lines show s.e.mean.

that which coincided with the period of highest number of writhes in a preliminary test.

In a number of studies, the levels of immunoreactive PGE_2 were measured and the writhes were counted in mice pretreated with various anti-inflammatory and analgesic agents at doses previously shown to inhibit writhing completely. The prostaglandin biosynthesis inhibitors (indomethacin, aspirin, ibuprofen) reduced both PGE_2 levels and the number of writhes (Table 1). Morphine inhibited writhing but had no statistically significant effect on PGE_2 levels (Table 1). Pooling data from all experiments (including all vehicle and drug-treated groups, except those treated with morphine) suggests that there is a relationship between the level of PGE_2 and the writhing response (Figure 2a,b). However, although there is a significant linear relationship between the number of writhes per mouse and the level of PGE_2 ($P = 0.0128$), the correlation ($r = 0.1838$) is weak (Figure 2a). The relationship between these two parameters is more obvious when the proportion of mice writhing, regardless of the number of writhes, is considered (Figure 2b). The higher the level of PGE_2, the higher the percentage of mice that writhe. However, it should be noted that in the experiments shown in Figure 2, mice were killed at different times after injection of zymosan in order to enable additional parameters to be measured (protein accumulation which peaks 1 h after zymosan injection; peptidoleukotriene levels which peak 15–30 min after injection, data not shown). This experimental variable could have obscured a stronger correlation between PGE_2 and the number of writhes.

The data from morphine-treated mice have been excluded from the analysis discussed above since they clearly exhibit a different pattern of response. All ten mice treated with 10 mg kg^{-1} s.c. morphine had PGE_2 levels > 0.055 ng per mouse, but none of them writhed. This was significantly different from the 131 mice in Figure 2 which also had PGE_2 levels > 0.055 ng per mouse, of which 115 writhed (Fisher's exact test, $P < 0.01$).

The ability of exogenous PGE_2 to reverse the inhibition of writhing induced by indomethacin was examined. In animals pretreated with indomethacin (1 mg kg^{-1}, orally) and injected with zymosan, no writhing occurred, as shown in Table 1 and previously (Doherty *et al.*, 1985a). Intraperitoneal injection of PGE_2 at a wide range of doses (2 ng to 2 μg per mouse) and times (3 h before to 10 min after zymosan) failed to

Table 1 The effects of various analgesic and anti-inflammatory agents on the writhing responses and increased levels of prostaglandin E_2 (PGE_2) induced by intraperitoneal injection of zymosan in mice

	Treatment	*Dose* (mg kg^{-1})	*Route*	n	*No. of writhes*	*% inhibition*	*PGE_2* (ng per mouse)	*% inhibition*
A	Vehicle	—	s.c.	9	4.6 ± 1.3	—	1.72 ± .24	—
	Morphine	10	s.c.	10	0	(100)*	1.17 ± .14	(32)
B	Vehicle	—	oral	20	10.1 ± 1.5	—	0.91 ± .11	—
	Ibuprofen	100	oral	5	0	(100)*	0.08 ± .02	(91)*
	Aspirin	100	oral	5	0	(100)*	0.08 ± .08	(91)*
	Indomethacin	1	oral	5	0	(100)*	0.07 ± .05	(92)*

*$P < 0.05$ cf vehicle controls. Results shown are means ± s.e.mean.
All compounds were administered 1 h before intraperitoneal injection of zymosan (1 mg in 0.5 ml). Writhes were counted 15–30 min after zymosan injection and animals were killed 30 min (Expt. A) or 60 min (Expt. B) after zymosan injection and peritoneal washings collected immediately.

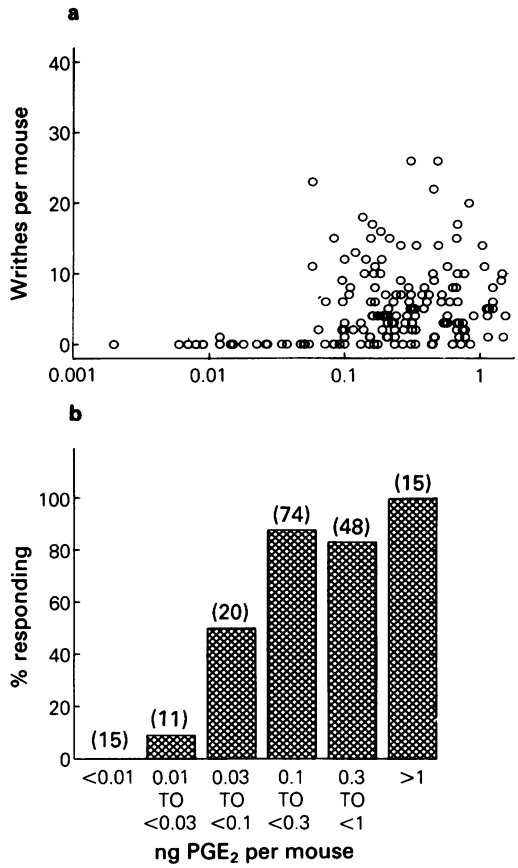


Figure 2 Prostaglandin E_2 (PGE_2) levels and the writhing response to zymosan. (a) The total number of writhes counted over a 15 min observation period and the peritoneal levels of PGE_2 are shown for each mouse. (b) The proportion of mice writhing at least once, grouped according to peritoneal PGE_2 levels. The numbers at the top of the columns indicate the total number of mice within that range of prostaglandin levels. Data from morphine-treated mice have been excluded.

restore the writhing response in indomethacin-treated mice (data not shown). In addition, PGE_2 failed to induce writhing in normal mice and failed to potentiate writhing in zymosan-injected mice (data not shown).

The failure of the above experiments to demonstrate reversal of the analgesic activity of indomethacin by administration of exogenous PGE_2 suggested that another cyclo-oxygenase product (or products), whose synthesis would also be inhibited by indomethacin, could be involved in mediating the writhing response. Therefore, g.c./m.s. was used to look at the levels of other cyclo-oxygenase products. PGE_2 and PGD_2 are positional isomers which produce derivatives with identical mass, and their oximine derivatives exist as syn and ante isomers. The g.c. resolves these four compounds with identical mass (524 atomic mass units) into three peaks, one of which is a mixture of one PGE_2 oxime isomer and one PGD_2 oxime isomer (peak 1 of Figure 3). The other two peaks contain only PGD_2 or only PGE_2 derivatives (peaks 2 and 3 respectively in Figure 3). Peritoneal fluid collected 15 min after zymosan injection clearly contained more PGE_2 than peritoneal fluid from non-injected mice (Figure 3). However, the levels of PGE_2 were very low (<4 ng per mouse) and close to the limits of detection of the assay, so they have not been quantified. These data are consistent with those obtained with the RIA assay (Table 1). PGD_2 levels are also found to be extremely low (<4 ng per mouse) and no difference between non-injected and zymosan-injected mice was seen. No TxB_2 was detected in either non-injected or zymosan-injected mice (i.e. <1 ng per mouse). In marked contrast, 6-keto-$PGF_{1\alpha}$, the non-enzymatic breakdown product of PGI_2, was found in readily measurable amounts in both non-injected and zymosan-injected mice (Figure 3). Fifteen minutes after zymosan injection, the levels were 20 times higher (228 ng per mouse) than in controls (9 ng per mouse) but fell rapidly thereafter (Figure 4), indicating the transient synthesis of a relatively large amount of PGI_2.

Since the g.c./m.s. data indicated that PGI_2 was the major cyclo-oxygenase product formed, an RIA assay for 6-keto-$PGF_{1\alpha}$ was used to examine the relationship between PGI_2 and the writhing response. The levels of 6-keto-$PGF_{1\alpha}$ detected by RIA were consistent with those detected by g.c./m.s. (Table 2). In order to measure the writhing response and also 6-keto-$PGF_{1\alpha}$ levels at a time close to their transient peak level, writhes were counted from 5 to 20 min after zymosan injection, the mice immediately killed, and peritoneal washing collected. The prostaglandin biosynthesis inhibitors examined (indomethacin, ibuprofen) reduced the levels of 6-keto-$PGF_{1\alpha}$ at doses effective in reducing the number of writhes (Table 2). In fact, these compounds reduced the 6-keto-$PGF_{1\alpha}$ to levels below those found in mice which had received no zymosan. In contrast, morphine and clonidine inhibited writhing without reducing the levels of 6-keto-$PGF_{1\alpha}$ (Table 2).

An analysis of the correlation between levels of 6-keto-$PGF_{1\alpha}$ and the writhing response is shown in Figure 5a,b. In this analysis, clonidine and morphine data are excluded. There is a significant linear relationship between the level of 6-keto-$PGF_{1\alpha}$ and the number of writhes ($P = 0.0001$) but with a poor correlation ($r = 0.4276$) (Figure 5a). The proportion of mice which writhed, regardless of the number of writhes, increased as the level of 6-keto-$PGF_{1\alpha}$ increased (Figure 5b). Clonidine and morphine-treated mice

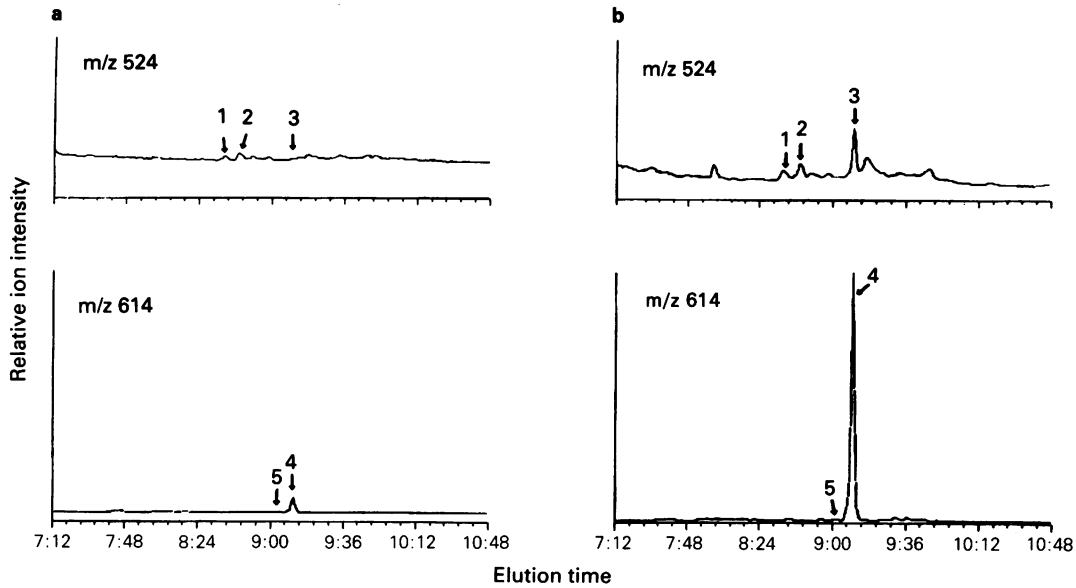


Figure 3 Gas chromatography/mass spectrometry analysis of the prostaglandins in the peritoneal lavage fluid from an untreated mouse (a) and a mouse injected with 1 mg of zymosan 15 min previously (b). 1 = prostaglandin D_2 (PGD_2) + PGE_2 (a mixture of isomers of the oxime derivatives), 2 = PGD_2, 3 = PGE_2, 4 = 6-keto-$PGF_{1\alpha}$, 5 = thromboxane B_2.

proved to be exceptions to this trend since, despite relatively high levels of 6-keto-$PGF_{1\alpha}$ (> 60 ng per mouse), a low proportion writhed (0/5, 2/5, respectively) compared to other mice with similar levels (130/144, $P < 0.05$ Fisher's exact test).

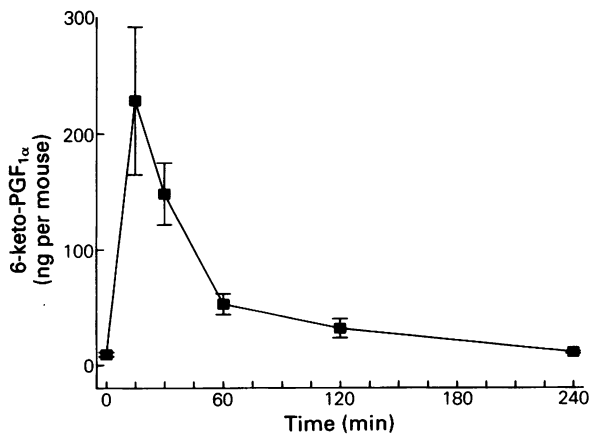


Figure 4 Time course of zymosan-induced prostaglandin I_2 (prostacyclin, PGI_2) synthesis. Groups of 5 mice were killed at various times after intraperitoneal injection of zymosan. The level of 6-keto-$PGF_{1\alpha}$ in the lavage fluid was measured by g.c./m.s. Means are shown with s.e.mean indicated by vertical lines.

In view of the correlation described above, the ability of PGI_2 to reverse the inhibition of writhing observed with indomethacin was examined. When injected intraperitoneally, 10 min after the i.p. injection of zymosan, PGI_2 reversed the analgesic effect of indomethacin (Figure 6). The effective doses of PGI_2 were in the range of those found in the peritoneal cavity of untreated zymosan-injected mice. Clonidine, morphine and pentazocine, but not ibuprofen, were able to block the reversal of the analgesic activity of indomethacin induced by i.p. administration of PGI_2 (2000 ng per mouse) (Table 3).

PGI_2 induced writhing when injected into otherwise untreated mice, although the incidence was low (6 of the 20 mice injected with 2000 ng of PGI_2 in 0.5 ml pH 10 carbonate buffer, writhed; none of the 20 mice injected with buffer alone writhed; $P < 0.05$, Fisher's exact test). In both of the above situations, the writhing induced by PGI_2 had a short latency to onset (< 1 min) and was of short duration (< 10 min).

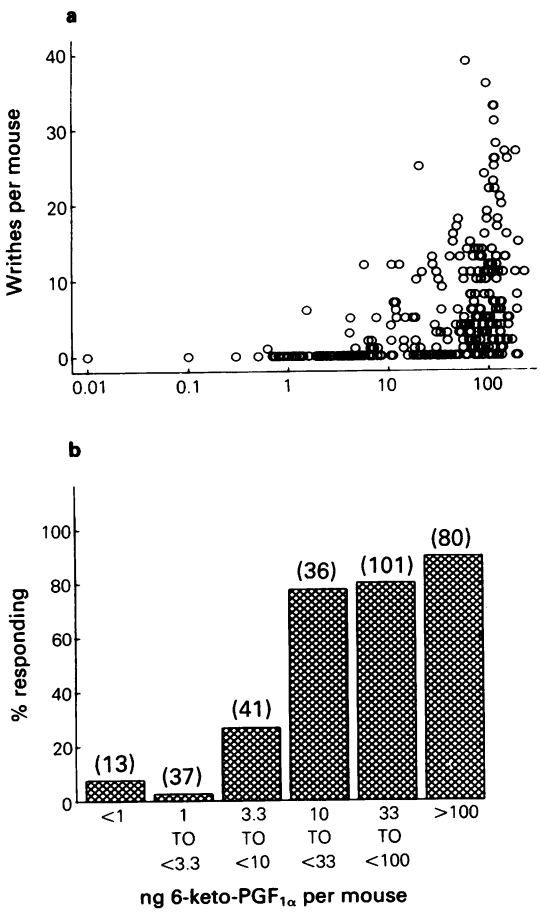
Discussion

The data described here confirm the usefulness of this model in the study of mediators of nociceptive responses and inflammation. However, it is clear that apparently trivial methodological details can be critically important. There is no obvious reason why

Table 2 The effects of various analgesic and anti-inflammatory agents on the writhing response and increased levels of 6-keto-$PGF_{1\alpha}$ induced by intraperitoneal injection of zymosan in mice

	Treatment	*Dose* ($mg\,kg^{-1}$)	*Route*	n	*No. of writhes*	*% inhibition*	*6-keto-$PGF_{1\alpha}$* (ng per mouse)	*% inhibition*
A	Vehicle	—	oral	7	9.6 ± 3.0	—	131.4 ± 11.6	—
	Ibuprofen	100	oral	7	.1 ± .1	(99)*	1.0 ± .1	(99)*
	Indomethacin	1	oral	7	1.6 ± 1.0	(83)*	3.4 ± .6	(97)*
	Aspirin	100	oral	7	5.0 ± 1.5	(48)	11.0 ± 2.1	(92)*
	Untreated (no zymosan)	—	—	4	0	—	6.6 ± 1.3	—
B	Vehicle	—	s.c.	10	23.5 ± 4	—	109.1 ± 11.9	—
	Clonidine	.1	s.c.	5	0	(100)*	149.7 ± 10.6	(−38)
	Morphine	10	s.c.	5	.4 ± .2	(98)*	96.2 ± 12.8	(12)
	Indomethacin	1	s.c.	5	1.8 ± 1.2	(92)*	5.2 ± 1.8	(95)*

*$P < 0.05$ cf vehicle controls. Results shown are mean ± s.e.mean.
All compounds were administered 1 h before intraperitoneal administration of zymosan (1 mg in 0.5 ml). Writhes were counted 5 to 20 min after zymosan injection and the animals were then killed to collect peritoneal fluid samples.



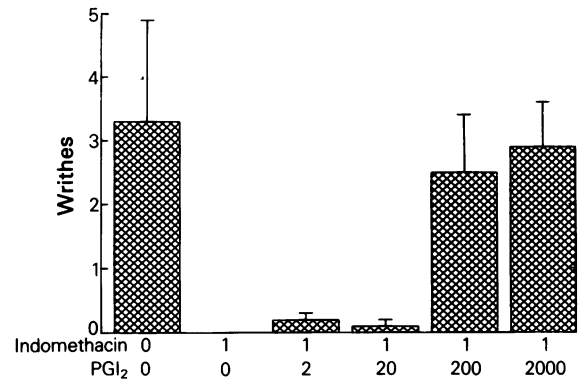


Figure 6 Reversal of the analgesic effect of indomethacin by prostaglandin I_2 (prostacyclin, PGI_2). Groups of mice were dosed orally with indomethacin ($1\,mg\,kg^{-1}$ orally) or water and, 1 h later, zymosan (1 mg) was administered i.p. PGI_2 (ng per mouse i.p.), or vehicle (carbonate buffer pH 10) was injected i.p. 5 min after the zymosan and the number of writhes counted over the next 15 min. The means of 10 mice per group are shown with s.e.mean indicated by vertical lines.

Figure 5 6-keto-Prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) levels and the writhing response to zymosan. (a) The total number of writhes counted over the period of 5–20 min after zymosan injection and levels of 6-keto-$PGF_{1\alpha}$ are shown for each mouse. (b) The proportion of mice writhing at least once, grouped according to peritoneal 6-keto-$PGF_{1\alpha}$ levels. The numbers at the top of the columns indicate the total number of mice within that range of prostaglandin levels.
Data from morphine- and clonidine-treated mice have been excluded.

Table 3 The ability of various analgesic and anti-inflammatory agents to block PGI_2-induced reversal of the analgesic effect of indomethacin

Treatment	*mg kg^{-1}*	*PGI_2*	*Writhes (± s.e.mean)*
Saline	—	−	0
Saline	—	+	3.7 ± 1.5
Clonidine	0.1 s.c.	+	0*
Saline	—	+	3.8 ± 1.1
Morphine	10 s.c.	+	0*
Saline	—	+	4.8 ± 2.1
Ibuprofen	50 s.c.	+	4.5 ± 1.6
Pentazocine	4 s.c.	+	0.4 ± 0.3*

*$P < 0.05$ cf saline s.c., PGI_2 i.p.
All mice were given indomethacin (1 mg kg^{-1} orally) 1 h before the intraperitoneal injection of zymosan (1 mg in 0.5 ml). Ten minutes after zymosan injection, the mice received either 0.05 ml of carbonate buffer pH 10 i.p. (−) or 2000 ng PGI_2 in 0.05 ml of buffer (+). Writhes were counted over the next 15 min. Test compounds were administered s.c. 30 min before zymosan injection. All groups consisted of 10 mice.

the writhing response should be so dependent on the volume of injection, but the phenomenon does explain the absence of a writhing response to zymosan in laboratories which use larger injection volumes (J. Humes, J. Cheng, personal communication).

G.c./m.s. analysis confirmed the RIA data (this report; Doherty *et al.*, 1985a) indicating the presence of increased amounts of PGE_2 following zymosan injection, although the levels were relatively low (<2 ng per mouse). PGD_2 and TxB_2 were not increased, but 6-keto-$PGF_{1\alpha}$ was increased 20 fold and reached levels (228 ng per mouse) which were much higher than those of the other prostanoids examined. Somewhat similar data have been reported by Pacholok *et al.* (1986). Therefore, it is clear that zymosan induced the rapid, but transient, synthesis of relatively large amounts of PGI_2 and a small amount of PGE_2. This synthetic activity is over and prostaglandin levels are falling towards normal before inflammatory cells arrive in the peritoneal cavity (Doherty *et al.*, 1985a). Resident peritoneal cells are therefore responsible for the synthesis of PGI_2 and PGE_2. Although mouse peritoneal macrophages synthesize both of these prostaglandins when stimulated with zymosan *in vitro*, they synthesize 3 to 4 times more PGE_2 than PGI_2 (Humes *et al.*, 1977; Bonney *et al.*, 1978). Rabbit peritoneum synthesized PGI_2 when stimulated *in vitro* with serum in which the complement system had been activated by incubation with zymosan (Rampart *et al.*, 1981). Peritoneum may, therefore, contribute a major portion of the PGI_2 observed in the present study. This latter study also emphasizes the role of the complement system in the response to zymosan, as has been also described in the rabbit (Jose *et al.*, 1983).

The effects of analgesic agents on prostaglandin levels enabled them to be classified into two groups: (A) the non-steroidal anti-inflammatory drugs (indomethacin, ibuprofen, aspirin) reduced the levels of both PGE_2 and 6-keto-$PGF_{1\alpha}$ at analgesic doses. This is consistent with the ability of these drugs to inhibit cyclo-oxygenase (Vane, 1971; Shen, 1978), the enzyme responsible for the synthesis of PGH_2, the immediate precursor of these prostaglandins (Flower, 1978). These data, however, do not indicate whether PGE_2, PGI_2, or both, are involved in the writhing response. (B) In contrast, morphine and clonidine did not significantly reduce the levels of prostaglandins at analgesic doses. This is consistent with the consensus of opinion that these drugs exert their analgesic effect via stimulation of opiate (Pert & Snyder, 1973) or α_2-adrenoceptors (Paalzow & Paalzow, 1976) respectively, and not by inhibition of prostaglandin synthesis.

The writhing response correlated equally well with the levels of PGE_2 and the levels of 6-keto-$PGF_{1\alpha}$. This is not surprising since the synthesis of both is dependent on the activity of cyclo-oxygenase (Flower, 1978). In order to determine whether these relationships were causal, the ability of i.p. administration of PGE_2 and PGI_2 to reverse the analgesic effects of an agent which reduces the levels of both, indomethacin, was examined. PGE_2 proved to be completely ineffective over a wide range of doses, including those found following zymosan injection, in reversing indomethacin analgesia, suggesting that it is not involved in mediating the writhing response to zymosan. This result was surprising, since other reports have described the ability of intraperitoneal doses of PGE_2 to induce writhing (Collier & Schneider, 1972; Dubinsky & Schupsky, 1984) and to reverse the ability of indomethacin to inhibit benzoquinone-induced writhing in mice (James & Church, 1978). No explanation for this discrepancy can be offered at present. In marked contrast to the lack of effect of PGE_2, PGI_2 completely reversed the analgesic effect of indomethacin at doses similar to those found in the peritoneal cavity of control zymosan-injected mice. Furthermore, PGI_2 induced writhing when injected into otherwise untreated mice. Similarly, Smith *et al.* (1985) found that i.p. administration of carbacyclin, a stable analogue of PGI_2, induced a writhing response that was resistant to inhibition by indomethacin. These data therefore unequivocally demonstrate that the correlation between the levels of PGI_2 and the writhing response is causal and that PGI_2 is an essential mediator in this model. It also seems possible that PGI_2 may directly stimulate the nociceptors in the

peritoneal cavity in addition to producing increased sensitivity to other stimuli. However, the possibility that PGI_2 is, in fact, potentiating the response to mediators induced by the trauma of injection, cannot be completely excluded.

The ability of PGI_2 to reverse analgesia was confined to analgesic agents which are cyclo-oxygenase inhibitors (indomethacin, ibuprofen); morphine clonidine and pentazocine retained their full activity even in the presence of exogenous PGI_2. Thus, this technique provides a convenient means of identifying compounds which exert analgesic activity by inhibition of prostaglandin synthesis at the site of the inflammation. It also demonstrates that, although cyclo-oxygenase inhibitors may have a central component to their analgesic activity in some systems (Ferreira *et al.*, 1978a; Okuyama & Aihara, 1985), their mechanism is entirely peripheral in zymosan-induced writhing.

In summary, the data described here directly confirm previous suggestions that PGI_2 is an important mediator of inflammatory pain and that cyclo-oxygenase inhibitors exert their analgesic activity by inhibiting PGI_2 synthesis at the site of the inflammation. It remains to be seen whether the same is true of other animal models of inflammatory pain and in inflammatory pain in man.

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