Correlation of desensitisation of platelet activating factor (PAF) receptors with intensity of inflammation and intestinal PAF content during experimental ileitis in guinea pig

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Abstract

Aim—To determine the kinetics of platelet activating factor (PAF) and prostaglandin E₂ (PGE₂) receptor desensitisation during intestinal inflammation induced by trinitrobenzenesulphonic acid (TNB) instillation and to study the relation between receptor regulation, inflammatory lesions, and PAF content of the gut wall.

Methods—Receptor desensitisation was assessed on isolated smooth muscle cells from the circular layer. PAF content of the intestinal wall was determined by thin layer chromatography and radioimmunoassay.

Results—After an acute inflammatory phase on day 1, subacute changes appeared in TNB instilled ileum, with a maximal intensity on day 6. In control animals, PAF 10 nM and PGE₂ 10 nM provoked a maximal contraction in the range of 24% of cell shortening. On days 1 and 3 after intestinal instillation of TNB, PAF induced contraction was not altered whereas the effect of PGE₂ was progressively desensitised (2 logM rightward shift of its concentration-response curve: C_{max} = 1 µM; p<0.01). Between days 4 and 6, the concentration-response curve of PGE₂ shifted by only 1 logM (p<0.05) whereas the curve of PAF induced contraction shifted by 2 logM (C_{max} = 1 µM; p<0.01). The PAF content of the ileal wall was maximal between days 3 and 5 (300 ng/mg tissue). On days 10 and 15, PAF and PGE₂ induced contractions were similar to those observed on day 1, and PAF content returned to basal.

Conclusion—Inflammation induced by TNB instillation triggers PAF and PGE₂ receptor desensitisation; this is dependent on the duration of inflammation and correlates with PAF content in the ileum. This receptor desensitisation may play a protective role by preventing overstimulation of intestinal smooth muscle cells.

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Keywords: platelet activating factor receptor; prostaglandin E₂ receptor; receptor desensitisation; intestinal inflammation; trinitrobenzenesulphonic acid; smooth muscle cells

Platelet activating factor (1-O-alkyl-2-O-acetyl-sn-3-glycerophosphorylcholine; PAF) plays an important role in inflammatory processes (for a review, see Braquet *et al*). At the level of the digestive tract, PAF has been recognised as an inflammatory mediator during ischaemic colitis, inflammatory bowel diseases (IBDs) such as Crohn's disease and ulcerative colitis, and experimental inflammation in animals. Indeed, high concentrations of PAF have been measured in stools of patients with Crohn's disease or ulcerative colitis.²⁻⁴ Biopsy samples of colonic and ileal mucosa from these patients contain increased amounts of PAF.⁵⁻⁸ During experimental inflammation induced by colonic instillation of trinitrobenzenesulphonic acid (TNB), large amounts of PAF are produced, as shown by *in vivo*^{9,10} and *in vitro*¹¹ experiments. Instillation of TNB into intestinal lumen has been developed by Morris *et al*¹² as a simple and reproducible model of chronic intestinal inflammation in rat. This model has since been adapted for guinea pig.^{13,14} The histological changes that it induces mimic those observed in Crohn's disease and ulcerative colitis and are characterised by a marked white cell infiltrate, submucosal fibrosis, and smooth muscle and mast cell hyperplasia. These lesions are maximal seven days after TNB instillation and recover progressively within two to three weeks.

Intestinal motility disturbances have been described in patients with IBD¹⁵ as well as in animals with experimental colitis.^{16,17} In rat, changes in colonic motility induced by intra-colonic TNB instillation are decreased by previous administration of a PAF receptor antagonist.¹⁸ In rabbit colon, PAF may participate in colonic dysmotility after intra-arterial infusion of PAF.¹⁹ A recent study has shown that intra-arterial administration of PAF increases the frequency with which giant migrating contractions appear after instillation of ethanol/acetic acid in dog colon.²⁰

We have previously shown that PAF receptors are present on the membrane of guinea pig intestinal smooth muscle cells.²¹ These receptors are desensitised after prolonged incubation of cells *in vitro* with high concentrations of PAF.²² Desensitisation of PAF receptors also occurs *in vivo* six days after intestinal instillation of PAF.²³ This desensitisation is characterised by a rightward shift of the concentration-response curve of PAF. Experimental evidence has been obtained from studies on human²⁴

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and rabbit^{25, 26} platelets, human neutrophils,^{27, 28} and rat Kupffer cells²⁹ that PAF receptors may be desensitised after prolonged exposure to PAF. In pathological conditions, membrane receptors may thus be desensitised to counteract excessive stimulation by large amounts of agonist.

In these experiments, we investigated the time course of desensitisation of PAF receptors after TNB instillation into the ileum of guinea pig. Since we previously observed that prostaglandin E₂ (PGE₂) mediates desensitisation of PAF receptors in smooth muscle cells,²² we also evaluated the influence of TNB induced inflammation on PGE₂ receptors over time. To evaluate the pathophysiological role of PAF receptor desensitisation further, we also correlated changes in PAF and PGE₂ induced contraction with the histological alterations in the intestinal wall and the content of PAF in the gut. To determine the specificity of the changes observed in the effect of PAF and PGE₂ on smooth muscle cells, we measured the effect of cholecystokinin (CCK) and acetylcholine (ACh) in the same experimental conditions, since they are not supposed to play any role as inflammatory mediators in the intestine.

Materials and Methods

ANIMALS AND EXPERIMENTAL INFLAMMATION

Healthy adult albino male guinea pigs (Interfauna, Loches, France) weighing 300–400 g were used. After a 24 hour fast during which water was available ad libitum, animals were submitted to laparotomy under aseptic conditions and deep anaesthesia (acepromazine 2 mg/kg and ketamine 80 mg/kg administered by intraperitoneal injection). The animals were divided into two groups. One group of 40 guinea pigs kept as controls were given 0.2 ml saline as a single injection. Saline was infused directly into the intestinal lumen through a thin hypodermic needle (external diameter 0.4 mm) 15 cm orally from the ileocaecal junction. The second group of 40 animals were given a single intraluminal injection of 0.2 ml 50% ethanol containing TNB, at a final dose of 80 mg/kg. TNB was instilled into the intestinal lumen through a thin hypodermic needle.

Animals of both groups were then placed in individual cages with water and food available ad libitum until they were killed. Five animals from the control and TNB treated groups were killed 1, 2, 3, 4, 5, 6, 10 and 15 days after surgery. Animals were bled under anaesthesia and laparotomy was performed. Three intestinal segments were removed from a position 5 cm proximal to the ileocaecal junction. The first sample was used to prepare isolated smooth muscle cells and evaluate cell contraction induced by agonists. The second sample was used to measure PAF concentrations in the ileal wall by two different analytical techniques, and the final one was used for histological studies.

CELL DISPERSION

Cell dispersion was achieved as previously described.^{21–23} Briefly, small muscle strips from the circular layer were incubated for two successive periods of 30 minutes at 31°C in

medium (132 mM NaCl, 5.4 mM KCl, 5 mM Na₂HPO₄, 1 mM NaH₂PO₄, 1.2 mM MgSO₄, 1 mM CaCl₂, 25 mM Hepes, 0.2% (w/v) glucose, 0.2% (w/v) bovine serum albumin, pH 7.4), bubbled with 95% O₂ and 5% CO₂ and supplemented with antibiotics, penicillin G 100 IU/ml and streptomycin 50 µg/ml, containing 0.25 IU/ml collagenase A, 0.36 mg/ml Pronase, and 0.4 mg/ml soyabean trypsin inhibitor. At the end of the second incubation, the medium was filtered and the partly digested muscle strips were washed four times with enzyme free medium. These strips were then transferred into fresh enzyme free medium and left to stand for 20 minutes to allow the muscle cells to disperse spontaneously under very slow mechanical agitation created by an oxygen current. Cells were harvested through a 500 µm nylon filter. Only those cells that had spontaneously dissociated in enzyme free medium were used for functional measurements.

Evaluation of cell contraction

Dispersed cells were usually studied within 30 minutes of dissociation. The density of the suspension was about 150 000 cells/ml. Aliquots of 250 µl cell suspension were added to 250 µl solution containing the agent to be tested, thereby ensuring rapid mixing, and then incubated for 30 seconds at 31°C. The reaction was stopped by addition of glutaraldehyde at a final concentration of 2.5%, which immediately fixes the cells. In control experiments, 250 µl medium alone was substituted for the test agent. To evaluate cell length, an aliquot of fixed cells was placed on a Malassez slide and the length of the first 50 entire cells randomly encountered in successive microscopic fields was measured. This method of measurement has been extensively validated over the last two decades in several laboratories (for a review see Makhoulouf³⁰). In our laboratory, it has been validated by assessing interoperator correlation of measurements of 450 resting smooth muscle cells ($r = 0.73$) and correlation between repeated measurements of the same cell samples by one operator (intraoperator variability; $r = 0.84$). Likewise, the reproducibility of contraction assessment has been assessed during blinded repeated measurements performed by two independent operators ($r = 0.77$).

Expression and analysis of results

The contractile response was defined as the percentage decrease in the average cell length of a population of smooth muscle cells treated with an agent in comparison with controls. At each experimental point, the decrease in cell length was determined using the following formula:

$$\Delta L = [(L_0 - L_x)/L_0] \times 100$$

where L_0 is the mean length of cells in the resting state and L_x the mean length of treated cells. The EC₅₀ (concentration inducing a half-maximal contraction) of each agonist was determined for each experiment on individual concentration-response curves for each sample. The mean EC₅₀ values for experiments performed at different time intervals were then calculated and compared by Student's *t* test.

To analyse the rightward shift of the concentration-response curve of PAF and PGE₂ obtained after instillation of TNB, we calculated the variance of the contraction induced by PAF or PGE₂ at various concentrations and evaluated the effect of pretreatment with saline or TNB as an independent factor. Using the General Linear Model, we evaluated a possible interaction between TNB instillation and PAF and PGE₂ concentrations. The finding of a positive interaction indicates that concentrations are influenced by TNB instillation. In this model, the mean contraction values were adjusted by the Bonferroni correction.

MEASUREMENT OF PAF CONTENT OF THE GUT WALL

Tissue PAF content was measured in ileum from saline and TNB treated guinea pigs as well as in ileum from untreated animals (day 0). Ileal segments (150 to 300 mg) were removed at various time intervals after intraluminal instillation of saline or TNB, weighed, immediately placed in liquid nitrogen and stored at -80°C until the day of treatment.

Lipid extraction

The Bligh and Dyer technique³¹ has been adapted for lipid extraction. Before lipid extraction, intestinal samples were rinsed in Tyrode buffer (137 mM NaCl, 2.7 mM KCl, 11.9 mM NaHCO₃, 0.42 mM NaH₂PO₄, 1 mM MgSO₄, 1 mM CaCl₂, 1 g/l glucose, 0.25% (w/v) bovine serum albumin, pH 7.4). They were then crushed and homogenised for three periods of 20 seconds at 4°C with a Polytron. After addition of 10 ml chloroform/methanol (50:50, v/v), the cellular homogenate was centrifuged (1000 rpm; five minutes; 10°C). Two phases could be easily distinguished: the organic phase (bottom of the tube) containing lipids was taken and stored. The aqueous phase (upper phase) and the tissue debris located between the two phases were resuspended in 4 ml chloroform/methanol and centrifuged (1000 rpm; five minutes; 10°C). At the end of this second centrifugation, the lipid phase was pooled with that obtained from the first extraction. Lipid extract was then evaporated using a rotary evaporator under vacuum and resuspended in 80% ethanol (1 ml). The rate of lipid recovery was assessed by adding a known concentration (6 nM) of [³H]PAF to the initial cellular homogenate. Preliminary experiments showed that 94.03 (3.06)% of [³H]PAF was recovered at the end of this lipid extraction procedure (mean (SEM)).

Purification of PAF

PAF contained in lipid extracts was purified by the technique proposed by Denisot *et al.*³² This method is based on polar extraction of the various lipids. Lipid extracts were placed on minicolumns of volume 1 ml with a maximal lipid capacity of 1 mg (Amprep Silica Si, Amersham, Les Ulis, France) and eluted under vacuum at a flow rate of about 0.2 ml/min, using a "Super-Separator" (Amersham), at room temperature. At the beginning of the procedure, the minicolumn was condi-

tioned with 2 ml methanol. Lipid samples were then loaded in 1 ml 80% ethanol. After adsorption, a first elution was performed with 2 ml dichloromethane to eliminate non-polar lipids. A second elution with 3 ml 60% ethanol was then performed to recover PAF. This fraction was dried using a rotary evaporator under vacuum and resuspended in 200 µl methanol. The rate of PAF recovery was evaluated by adding a known concentration of [³H]PAF to the column. Preliminary studies have shown that the rate of PAF recovery after elution is 91.95 (2.13)% (mean (SEM), n = 5).

Quantification of PAF in lipid extracts

Thin layer chromatography—Samples (2 ml) of purified extracts at various dilutions (1:8; 1:16; 1:32) (10⁻² M) of PAF solutions were applied to the same silica gel plate (DC-Alufolien, Kieselgel 60; Merck, France). After the plate had been dried, lipids migrated along it in a solvent system of chloroform/methanol/32% acetic acid (5:3:1, by vol). Two methods of visualisation were used. (1) A specific reaction was produced by spraying Dragendorff reagent (mixture of potassium iodide and bismuth subnitrate) on the plate. Choline-containing lipids appeared in a few minutes as orange-red spots; these were relatively unstable, the intensity decreasing with time. (2) A non-specific carbonisation of organic compounds was produced by spraying H₂SO₄/methanol (50:50, v/v) on the plate, which was then heated at 150°C for 10 minutes.

After migration and spraying, the position of PAF on the plate was defined by its R_F (retention factor). The identification of PAF contained in purified extracts was performed by comparing the R_F spots with that of PAF standards. PAF was quantified by fluorimetric detection at 600 nm (CS 930 scanning densitometer; Shimadzu Corp, Kyoto, Japan) using a tungsten lamp. The relation between the quantity of PAF and the area of the spot was linear between 0.33 and 1.3 µg/2 µl on a calibration curve. The intensity of the spots defined the concentration of PAF contained in the extracts. For this analytical technique, the threshold of PAF detection was 0.3 µg for 2 µl purified extract.

Radioimmunoassay—PAF contained in the lipid extracts purified on minicolumns was also quantified by radioimmunoassay ([³H]PAF scintillation proximity assay (SPA); Amersham), the detection threshold of which is 20 pg. The assay is based on the competition between unlabelled ligand and a fixed quantity of radiolabelled ligand for a limited number of binding sites on a specific antibody. With fixed amounts of antibody and radiolabelled ligand, the amount of radiolabelled ligand bound by the antibody is inversely proportional to the concentration of unlabelled ligand.

Statistical analysis

Differences in PAF concentrations between samples from TNB treated animals and controls were analysed by variance analysis adjusted by the Bonferroni correction.

Figure 1 Normal histological pattern of the ileum six days after instillation of saline into the lumen (haemalum/eosin stain; original magnification $\times 6.3$).

Figure 2 Acute inflammation induced by trinitrobenzenesulphonic acid (TNB) instillation into the lumen of guinea pig ileum. On day 1 after the instillation, the lesions are mainly characterised by the presence of oedema in the submucosa and the infiltration of mononuclear cells and neutrophils and mucosal erosions (haemalum/eosin stain; original magnification $\times 16$).

Figure 3 Second phase of the inflammation provoked by trinitrobenzenesulphonic acid (TNB) in guinea pig ileum. Villi are destroyed. The mucosa is largely eroded by deep ulcerations. Infiltration into the submucosa is maximal (day 3) (haemalum/eosin stain; original magnification $\times 6.3$).

HISTOLOGICAL STUDIES

Ileal samples from saline or TNB treated animals were fixed immediately after removal in 10% buffered formalin. After dehydration through graded strengths of ethanol, intestinal samples were cleared in toluene solution and then embedded in Histomed standard (Labo Moderne, Paris, France). Transverse sections of 5 μm thickness were pasted with albuminous water (0.5%) on slides warmed to 45°C. Intestinal sections were then deparaffinised in toluene, rehydrated through a series of baths containing increasing concentrations of ethanol and then immersed in water. For each sample, two slides were prepared: one was stained with haemalum/eosin to study the population of inflammatory cells, and the other with periodic acid/Schiff (PAS) to localise mucopolysaccharides containing unsubstituted 1,2-glycol radicals.

The intensity of intestinal inflammation was assessed by scoring changes, such as ulceration, mucous cell depletion, mucosal atrophy, submucosal oedema, infiltration by inflammatory cells, and vascular dilatation, observed on inflamed specimens. The presence of one of these parameters was scored as 1 and its absence as 0. The sum gives an inflammatory score which was considered to be an index of intensity of inflammatory changes. Scores could thus range between 0 and 6 (presence of all changes). Histological scores were obtained during blinded examination of histological slices by the same pathologist. Statistical analysis was carried out using variance analysis.

MATERIALS

PAF, PGE₂, and TNB were purchased from Sigma (St Quentin Fallavier, France). Collagenase type V, Pronase, and soybean trypsin inhibitor were obtained from Boehringer Mannheim (Meylan, France). Penicillin G and streptomycin were from Specia (Paris, France). CCK octapeptide, ACh, and all other reagents were obtained from Sigma.

Results

KINETICS OF HISTOLOGICAL CHANGES IN ILEUM OF TNB TREATED GUINEA PIGS

Whatever the day of death of the animals, no macroscopic or microscopic intestinal damage was detected after saline treatment (fig 1).

In contrast, acute inflammation was observed on the days after intraluminal instillation of TNB. Macroscopically, the ileum appeared congested with haemorrhages and ulcerations from day 1 until day 3. Muscular layers appeared thickened. From day 4 to day 15, small haemorrhages were observed and the ileum wall appeared thickened. The lumen was dilated.

Microscopically, from day 1 until day 3 after TNB treatment, ulcerations exceeding the submucosa were observed with submucosal oedema. Neutrophils and macrophages infiltrated the submucosa and the circular muscle layer. Blood vessels were distended. Mucus cell depletion occurred in some glandular crypts and villi (fig 2). From day 4 to day 6, the number of erosions and/or deep ulcerations exceeding the submucosa increased, involving

Figure 4 Subacute phase of inflammation with fibrosis in the submucosa. Ulcerations are less deep than in the earlier stages of inflammation (day 6) (haemalum/eosin stain; original magnification $\times 6.3$).

Figure 5 Inflammatory index characterising morphological changes in intestinal wall after instillation of trinitrobenzenesulphonic acid (TNB). Five animals were killed at each time point and the inflammatory index was measured for each of the samples. Data are shown as mean (SEM). The inflammatory index is statistically significantly different from that on day 0, except on days 4, 10, and 15.

about 50% of the mucosal surface. Submucosal oedema worsened. Inflammatory cells infiltrated the submucosa, with a large number of mononuclear cells, neutrophils and macrophages. Dilatation of blood vessels remained considerable. Several microhaemorrhages were present in the submucosa (fig 3).

From day 10 to day 15, the inflammatory lesions progressively healed. The most charac-

teristic changes were multifocal erosions. Moderate oedema of the submucosa was still present. The intensity of inflammatory infiltration decreased, but a large number of fibroblasts were present in the submucosa where fibrosis was observed (fig 4).

The intensity of the morphological changes in the ileum during the course of TNB induced inflammation, characterised by the inflammatory index, was maximal after 6 days (fig 5): the index was 4.66 (0.49). By day 15, the inflammatory index was 1.82 (0.32) and was no longer significantly different from the index on day 0 (0.32 (0.16)).

KINETICS OF RECEPTOR DESENSITISATION AFTER TNB TREATMENT

PAF receptor desensitisation

In control animals, after instillation of saline, the characteristics of PAF induced contraction were similar no matter when the animals were killed. Maximal contraction induced by PAF ranged between 22.5 (3.1) and 24.3 (1.5)% of resting cell length and was obtained at 10 nM PAF. The EC_{50} was 18 (4) pM; it did not significantly change over time, and was similar to that previously observed in freshly dispersed cells.^{22 23}

In TNB treated animals, the characteristics of cell contraction induced by PAF were modified depending on the time between instillation of TNB and killing of the animals. When the animals were killed between days 1 and 3, no modification of PAF induced contraction was observed. C_{max} was 10 nM and EC_{50} for PAF ranged between 4 (0.5) and 60 (4) pM and were not statistically significantly different from those observed in control animals (n = 5). On day 4, the concentration-response curve of PAF shifted slightly towards higher PAF concentrations, by 1 logM. The C_{max} for PAF was 100 nM and the EC_{50} was 400 (20) pM, significantly different from controls (p<0.05) (n = 5). On days 5 and 6, the concentration-response curve of PAF had shifted by 2 logM towards higher concentrations. The C_{max} for PAF was 1 μ M and the EC_{50} ranged between 600 (60) and 950 (70) pM (p<0.01) (n = 5). On days 10 and 15, the characteristics of PAF induced contraction were not different from those observed in cells from control animals (C_{max} = 10 nM; EC_{50} = 8 (0.3) and 40 (6) pM) (n = 5) (table 1; fig 6).

Independently of the interval between TNB instillation and the death of the animal, the

Table 1 Characteristics of contraction induced by platelet activating factor (PAF) and prostaglandin E_2 (PGE_2) after intestinal inflammation provoked by trinitrobenzenesulphonic acid

Day of inflammation	PAF			PGE_2		
	Maximal contraction	C_{max} (nM)	EC_{50} (pM)	Maximal contraction	C_{max} (nM)	EC_{50} (pM)
1	14.9 (0.4)	10	20 (3)	12.6 (2.2)	10	6 (0.4)
2	14.4 (0.8)	10	4 (0.5)	14.1 (2.3)	100*	50 (5)
3	16.0 (0.6)	10	60 (4)	13.3 (5.9)	1000**	900 (40)
4	14.3 (1.9)	100*	400 (20)	13.4 (1.3)	100*	200 (60)
5	13.1 (1.1)	1000**	600 (60)	12.8 (2.8)	100*	50 (3)
6	15.5 (2.7)	1000**	950 (70)	15.8 (2.7)	100*	200 (50)
10	14.4 (0.9)	10	8 (0.3)	14.6 (0.1)	10	20 (6)
15	15.8 (2.1)	10	40 (6)	15.7 (1.8)	10	10 (3)

Where applicable, values are mean (SEM) (n=5). C_{max} , concentration of agonist inducing a maximal effect; EC_{50} , concentration of agonist inducing 50% of the maximal effect. Values significantly different from those obtained in cells from control animals: *p<0.05, **p<0.01.

Figure 6 Kinetics of desensitisation of platelet activating factor (PAF; solid circles) and prostaglandin E_2 (PGE_2 ; open circles) receptors of smooth muscle cells of the circular layer, after intraluminal instillation of trinitrobenzenesulphonic acid (TNB) into guinea pig ileum (mean (SEM) of samples obtained at each time point from five different animals). The concentration of PAF and PGE_2 inducing maximal contraction (C_{max}) of the cells is expressed in logM and plotted against time elapsed between TNB instillation and the killing of the animals. Values of C_{max} significantly different from control animals treated with saline: * $p < 0.05$; ** $p < 0.01$.

magnitude of the maximal contraction induced by PAF was significantly reduced by 30%, compared with that induced by PAF in cells from saline treated animals ($p < 0.01$) (cf table 1).

PGE_2 receptor desensitisation

In control animals, after instillation of saline, the characteristics of PGE_2 induced contraction were similar no matter when the animals

were killed. The maximal contraction induced by PGE_2 ranged between 21.9 (2.3) and 24.7 (3.9)% of resting cell length and was obtained at 10 nM PGE_2 . The EC_{50} for PGE_2 was 8 (4) pM, did not significantly change over time, and was similar to that previously observed in freshly dispersed cells.^{22 23}

In TNB treated animals, the characteristics of cell contraction induced by PGE_2 were modified depending on the time between TNB instillation and the killing of the animals. When they were killed on day 1 after TNB instillation, no modification of PGE_2 induced contraction was observed. C_{max} was 10 nM and EC_{50} for PGE_2 was 6 (0.4) pM; these values were not statistically different from those observed in control animals ($n = 5$). On day 2, the concentration-response curve of PGE_2 had shifted slightly towards higher PGE_2 concentrations, by 1 logM. C_{max} of PGE_2 was 100 nM and EC_{50} was 50 (5) pM, significantly different from controls ($p < 0.05$) ($n = 5$). On day 3, the concentration-response curve of PGE_2 had shifted by 2 logM towards higher concentrations. C_{max} of PGE_2 was 1 μ M and EC_{50} was 600 (60) and 900 (40) pM ($p < 0.01$) ($n = 5$). On day 4, the characteristics of PGE_2 induced contraction were similar to those observed on day 2, with a 1 logM shift of the concentration-response curve of PGE_2 and remained stable until day 6 ($C_{max} = 100$ nM; EC_{50} ranging from 50 to 200 pM) ($n = 5$). On days 10 and 15, the characteristics of PGE_2 induced contraction were not different from those observed in cells from control animals ($C_{max} = 10$ nM; $EC_{50} = 20$ (6) and 10 (3) pM) (table 1; fig 6).

Table 2 Characteristics of cell contraction induced by cholecystokinin (CCK) and acetylcholine on intestinal smooth muscle cells of guinea pig: comparison between saline and trinitrobenzenesulphonic acid (TNB) treated animals

	CCK 8			Acetylcholine		
	Maximal contraction	C_{max} (nM)	EC_{50} (pM)	Maximal contraction	C_{max} (nM)	EC_{50} (pM)
Fresh cells	21.9 (1.8)	10	20 (1.3)	23.9 (2.7)	1000	1.4 (0.5)
Saline	23.0 (2.5)	10	5.0 (0.6)	26.3 (2.2)	1000	1.0 (0.3)
TNB Day 1	14.7 (2.3)	10	10.0 (1.4)	15.1 (1.8)	1000	1.0 (0.5)
TNB Day 2	15.5 (2.6)	10	7.2 (1.5)	15.5 (2.4)	1000	1.3 (0.8)
TNB Day 3	16.3 (3.1)	10	5.0 (1.2)	14.0 (3.2)	1000	2.0 (1.2)
TNB Day 4	15.7 (2.8)	10	10.2 (1.4)	15.8 (1.8)	1000	1.1 (0.4)
TNB Day 6	15.9 (1.7)	10	10.1 (1.4)	15.5 (2.9)	1000	1.0 (0.8)
TNB Day 10	17.4 (2.7)	10	5.8 (1.3)	18.6 (3.2)	1000	1.3 (0.5)
TNB Day 15	17.8 (2.6)	10	7.1 (1.5)	18.9 (2.1)	1000	1.4 (0.8)

Where applicable, values are mean (SEM) ($n=5$).

Table 3 Measurement of platelet activating factor (PAF) content in the ileal wall by thin layer chromatography (TLC) and radioimmunoassay (RIA) in saline and trinitrobenzenesulphonic acid (TNB) treated guinea pigs

Day of inflammation	Ileal PAF content after saline treatment (ng/mg tissue; TLC)	Ileal PAF content after TNB treatment (ng/mg tissue)	
		TLC	RIA
Controls (day 0)	109 (13)	109 (13)	123 (17)
1	195 (19)*	165 (22)	187 (11)
2	163 (3)	237 (6)**†	240 (22)*
3	261 (32)***	348 (31)***†	300 (10)**
4	174 (18)*	295 (28)***†	317 (24)**
5	130 (27)	337 (49)***††	322 (43)**
6	132 (26)	290 (32)***††	267 (67)*
10	113 (9)	200 (9)**†	192 (35)
15	109 (16)	215 (30)*†	237 (16)*

Values are mean (SEM) (triplicate experiments on samples from five different animals).

Values significantly different from those measured as day 0: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Values significantly different from those measured on the same day, in samples from saline treated animals (applies only to TLC measurements): † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$.

Figure 7 Time relationship of platelet activating factor (PAF) receptor desensitisation in intestinal smooth muscle cells (solid circle) after trinitrobenzenesulphonic acid (TNB) instillation and PAF content in the ileum, after intraluminal instillation of saline (open squares) or TNB (solid squares) in guinea pig ileum. PAF content was evaluated by thin layer chromatography on the various days after TNB treatment (mean (SEM) for samples obtained at each time from five different animals). The concentration of PAF inducing a maximal cell contraction did not change over time in saline treated animals and is not shown. Statistically significant differences are quoted as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for comparison between TNB treated animals and values at D0; ° $p < 0.05$, °° $p < 0.001$ for comparison between saline and values at D0; † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$ for comparison between TNB and saline treated animals at the same time interval from surgery. On day 1 after intraluminal instillation of TNB, we observed a moderate increase in PAF content of the gut wall (165 (22) ng/mg tissue) (NS) (table 3). Three days after TNB treatment, PAF content was maximal in injured ileum (348 (31) ng/mg tissue) ($p < 0.001$), and was significantly higher than that measured on day 3 in ileum from saline treated animals ($p < 0.01$). The PAF content remained at this high level for the next 2 days (days 4 and 5). From day 6, it decreased and returned to basal level by day 10 (200 (9) ng/mg tissue). On day 15, the amount of PAF in the ileum from TNB treated animals increased again (215 (30) ng/mg tissue) and was significantly higher than that measured in untreated animals (day 0) (109 (13) ng/mg tissue) ($p < 0.05$).

As for PAF and independently of the time between TNB instillation and the death of the animal, the magnitude of the maximal contraction induced by PGE_2 was significantly reduced by 30%, compared with that in cells from saline treated animals ($p < 0.01$) (cf table 1).

CELL CONTRACTION INDUCED BY CCK8 AND ACh
CCK8 and ACh induced a contraction of intestinal smooth muscle cells from control animals with characteristics similar to those previously observed in freshly dispersed cells (table 2). In TNB treated animals, the concentration-response curves of ACh and CCK8 were not modified (table 2). However, independently of the time between TNB instillation and death of the animal, the magnitude of cell contraction was decreased by about 30% as observed for PAF and PGE_2 .

PAF CONTENT IN INTESTINAL WALL AFTER TNB INSTILLATION

Thin layer chromatography

A calibration curve was established by measuring the PAF content of standard solutions for each series of dosages. The calibration curves obtained were linear and the correlation coefficient (r) always varied between 0.996 and 1. PAF content of ileum fragments from

untreated animals (day 0) was 109 (13) ng/mg tissue ($n = 8$).

In ileum from saline treated animals, PAF content of the intestinal wall increased 24 hours after surgery to 195 (19) ng/mg tissue ($p < 0.05$). It then increased further from day 1 to day 3 (to 261 (32) ng/mg tissue) ($n = 3$) ($p < 0.001$) compared with untreated animals. Four days after saline treatment, PAF concentrations in the gut wall decreased (174 (18) ng/mg tissue) and returned to baseline values at day 15 (109 (16) ng/mg tissue) ($n = 3$) (table 3; fig 7).

Radioimmunoassay

Using the radioimmunoassay technique, we found that on day 1 after TNB treatment, the PAF content of the intestinal wall was not statistically significantly different in TNB treated animals (187 (11) ng/mg tissue) from that in controls (123 (17) ng/mg tissue) (table 3). On day 2, it progressively increased until day 5 in TNB treated animals (322 (43) ng/mg tissue) ($p < 0.05$). It then decreased from day 6 (267 (67) ng/mg tissue) but still remained above that in controls on day 10 (192 (35) ng/mg tissue) (table 3). Again, it had increased moderately on day 15 compared with that on day 0.

Discussion

In this study, we describe the time course of intestinal lesions provoked by an intraluminal instillation of TNB into guinea pig ileum. The inflammation triggered by TNB induces desensitisation of PAF and PGE_2 receptors and an increase in PAF content of the intestinal wall. These changes depend on the time elapsed between TNB instillation into the intestinal lumen and the death of the animals.

Changes in cell contraction observed after TNB instillation were of two types. (1) The capacity of smooth muscle cells to contract was altered by the inflammation since the magnitude of the maximal contraction induced by all the agents tested was decreased by about 30%. (2) Desensitisation of the contracting effect of PAF and PGE_2 was characterised by a rightward shift of the concentration-response curve towards higher concentrations. The effect of inflammation on cell contractility was unspecific as it affected in the same way the contraction induced by ACh and CCK as well as that induced by mediators involved in the control of inflammatory processes such as PAF and PGE_2 . Other studies have also shown modification of the amplitude of smooth muscle cell contraction in inflamed gut. Indeed, Snape *et al*¹⁵ observed a decrease in maximal tension of the colonic circular layer from patients with IBD after stimulation with betanecol. In vivo, disturbances of colonic motility, provoked in rat by TNB, are characterised by a decrease in myoelectrical activity and an increase in duration of the contractions.¹⁹ Recently, it was shown that ileitis induces desensitisation of the effects of carbachol and histamine on ileal circular strips in guinea pig.³³

In contrast, the desensitisation of the contractile effect of PAF and PGE_2 is specific to

these inflammatory mediators. We observed in this study changes in the capacity of PAF and PGE₂ to elicit contraction of smooth muscle cells that were similar to those we found previously when incubating cells in vitro with high concentrations of these agonists.²² In those in vitro studies and in the present in vivo experiments, the desensitising effect was limited to PAF and PGE₂ and did not affect the contraction induced by ACh or CCK. We concluded from the previous experiments that this desensitisation affected the receptors themselves, despite the fact that their pharmacological properties were not altered in inflamed cells.²³

Receptor desensitisation is often the consequence of a prolonged stimulation of membrane receptors by agonists and may function to prevent overstimulation of cells. This hypothesis is supported by the present observation that maximal desensitisation of PAF receptors was observed six days after TNB instillation and was preceded by three days of dramatic increase in the amount of PAF in the gut wall. Moreover these results are consistent with our in vitro experiments, which showed that desensitisation of PAF receptors is triggered by PAF itself.²² This hypothesis is further supported by our previous observation that an antagonist of PAF receptors prevents desensitisation of these receptors and decreases the intensity of inflammatory lesions in the ileum.²³ Similar results were obtained by others using experimental colitis.⁹ These experimental observations clearly show that desensitisation of PAF receptors during experimental ileitis is linked to the intensity of inflammation and is under the control of regulatory processes that also participate in the control of inflammation itself.

Among these regulatory mechanisms, a role for PGE₂ was suggested by in vitro experiments that showed that PAF receptor desensitisation in isolated intestinal smooth muscle cells was mediated by PGE₂.²² Indeed, PGE₂ may be involved as a protective agent of the cells during TNB induced inflammation.³⁴ A few studies in rat have shown that synthesis of PGE₂ occurs 48 hours after inflammation induced by TNB.^{10, 35} In the present study we observed that desensitisation of PGE₂ receptors occurs two days after TNB instillation in guinea pig ileum. Since we previously showed that PGE₂ receptor desensitisation is provoked by PGE₂ itself but not by PAF,²² we can assume that the amount of PGE₂ in the gut wall is able to increase dramatically during the first few days of intestinal inflammation. PAF content then also increases and both mediators may thus play a role in the desensitisation of PAF receptors, which occurs two days later. PGE₂ may thus facilitate the desensitising effect of PAF. However, the interaction of PAF and PGE₂ in the regulation of intestinal inflammatory processes may be more complex, as a recent study has shown that PAF may activate inducible cyclo-oxygenase and thus stimulate the synthesis of arachidonic acid metabolites.³⁶ Moreover, PGE₂ is able to downregulate PAF receptor expression by increasing concentrations of intracellular cAMP in monocytes.^{37, 38}

In vivo, the role of PGE₂ in controlling the desensitisation of PAF receptors during experimental ileitis has been shown in a previous report from our laboratory.²³ In these experiments, indomethacin, which inhibits the activity of cyclo-oxygenase and the production of prostaglandins, was able to prevent desensitisation of PAF receptors on day 6 of inflammation produced by TNB.

The kinetics of the inflammatory changes induced by TNB instillation into the ileum observed here show that experimental ileitis is characterised by two successive phases: the early phase is acute inflammation characterised by submucosal oedema and vascular dilatation; this is followed by a subacute or chronic phase characterised by the presence of submucosal fibrosis. These histological changes are consistent with those previously described in the literature.³⁹ These lesions are similar to those of Crohn's disease. The demonstration of a link between intensity of inflammation and desensitisation of receptors of various mediators involved in the control of inflammatory processes indicates that the modulation of these regulatory pathways could be of clinical interest. Indeed, drugs acting on such targets could have an effect on the course of symptoms of IBD or on some of their aspects, such as the disturbances of gut motility, that may participate in the pathogenesis of diarrhoea in patients with IBD. Such drugs could also improve inflammatory lesions in both acute and chronic phases of IBD. Since we observed a prolonged increase in PAF content of the intestinal wall, even on days 10 and 15 when desensitisation of PAF receptors had disappeared, we assume that desensitisation of receptors may be an initial protective mechanism of cells against overstimulation and may disappear in a more chronic situation when other defence mechanisms may contribute to cell protection.

In conclusion, we have shown that intestinal inflammation induced by intraluminal instillation of TNB into guinea pig ileum triggers a time dependent PAF and PGE₂ receptor desensitisation. This receptor desensitisation occurs during the subacute phase of inflammation and is consecutive to the synthesis or release of large amounts of PAF and PGE₂ in the gut wall. Modulation of this receptor desensitisation may also result in improvement of the natural course of inflammation.

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