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Delayed-type hypersensitivity-induced increase in vascular permeability in the mouse small intestine: inhibition by depletion of sensory neuropeptides and NK_1 receptor blockade

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1 This study investigates the effects of capsaicin-induced depletion of sensory neuropeptides and of neurokinin$_1$ (NK_1) receptor blockade on delayed-type hypersensitivity (DTH)-induced changes of vascular permeability in the small intestine of the mouse.

2 The DTH reaction in the small intestine was elicited by dinitrofluorobenzene (DNFB)-contact sensitization followed by oral dinitrobenzene sulphonic acid (DNBS) challenge. To assess vascular leakage the accumulation of the plasma marker, Evans blue (EB), was measured 2, 24 and 48 h after the challenge.

3 The small intestinal DTH reaction was characterized by a significant increase in vascular permeability 24 h after the challenge of previously sensitized mice when compared to vehicle-sensitized mice ($P<0.05$, ANOVA). Capsaicin-induced depletion of sensory neuropeptides, two weeks before the sensitization, completely inhibited the DTH-induced increase in small intestinal vascular permeability at 24 h ($P<0.05$, ANOVA). Vehicle/control: 108.2 ± 8.6 ng EB mg^{-1} dry weight; vehicle/DTH 207.8 ± 25.1 ng EB mg^{-1} dry weight; capsaicin/control: 65.8 ± 11.9 ng EB mg^{-1} dry weight; capsaicin/DTH: 84.3 ± 7.6 ng EB mg^{-1} dry weight.

4 The tachykinins, substance P and neurokinin A (1.5 to 50×10^{-11} mol per mouse, i.v.), induced an increase in vascular leakage in the small intestine of naive mice. The specific NK_1 receptor antagonist, RP67580 (10^{-9} mol per mouse, i.v.) was the most effective in reducing the substance P-induced plasma extravasation when compared with other NK receptor antagonists, FK224 and FK888.

5 Treatment of DNFB-sensitized mice with RP67580 (10^{-9} mol per mouse, i.v.) immediately before and 1 h after the DNBS challenge resulted in a significant reduction of the DTH-induced increase in vascular permeability at 24 h (vehicle/control: 107.5 ± 8.8 ng EB mg^{-1} dry weight; RP67580/control: 95.4 ± 5.4 ng EB mg^{-1} dry weight; vehicle/DTH: 206.6 ± 22.6 ng EB mg^{-1} dry weight; RP67580/DTH: 132.6 ± 13.6 ng EB mg^{-1} dry weight, $P<0.05$, ANOVA).

6 These results suggest that sensory nerves are involved in the development of small intestinal DTH reactions in the mouse. NK_1 receptors could play an important role in the initiation of the DTH-induced changes in vascular leakage.

Keywords: Small intestinal vascular permeability; delayed-type hypersensitivity; mouse small intestine; sensory neuropeptides; capsaicin; tachykinins

Introduction

It has been proposed that delayed-type hypersensitivity (DTH) reactions in the gastrointestinal tract resemble some of the pathological features of inflammatory bowel diseases (IBD) in man (Kagnoff, 1987). These pathological conditions of inflammation are characterized by an increased T cell activation and abnormalities in intestinal T cell function (Fais *et al.*, 1987; MacDonald *et al.*, 1990; Schreiber *et al.*, 1991). It has been suggested that DTH reactions require lymphocyte (T cell) and mast cell activation (Askenase & van Loveren, 1983). Both cell types are sensitive to sensory neuropeptides. In the periphery, sensory efferent neurones innervate the gastrointestinal tract and are found in close association with lymphocytes and mucosal mast cells (Polak, 1978; Skofitsch *et al.*, 1985; Stead *et al.*, 1987a,b; 1989; Arizono *et al.*, 1990; Dvorak *et al.*, 1992). In addition, neuroendocrine modulation of the intestinal immune system during inflammatory reactions has been demonstrated (Bienenstock *et al.*, 1988; Shanahan & Anton, 1988; Koch *et al.*, 1991). The described effects of neuropeptides on particular immunological functions are variable and frequently opposing: e.g. substance P enhances mitogen-stimulated lymphocyte proliferation (Stanisz *et al.*, 1986; Scicchitano *et al.*, 1988), whereas vasoactive intestinal peptide, somatostatin and calcitonin gene-related peptide inhibit mitogen-stimulated lymphocyte proliferation (Stanisz *et al.*, 1986; Umeda *et al.*, 1988). Substance P has the ability to activate mucosal mast cells and substance P-induced inflammatory responses have been shown to be mast cell-dependent (Joos *et al.*, 1993; Shanahan *et al.*, 1985; Matsuda *et al.*, 1989; Yano *et al.*, 1989; Joos & Pauwels, 1993). The role of sensory neuropeptides in cell-mediated DTH reactions has been studied in the skin using capsaicin to deplete sensory nerves (Girolomoni & Tigelaar, 1990). Capsaicin is a neurotoxin and induces an initial excitation with a massive release of mediators of unmyelinated sensory C fibres rapidly followed by a desensitization phase with depletion of neuropeptides (Jancso *et al.*, 1967; Buck & Burks, 1986).

In the present study, a DTH reaction was elicited in the small intestine of mice with dinitrofluorobenzene (DNFB) as

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the contact sensitizing hapten followed by an oral challenge with dinitrobenzene sulphonic acid (DNBS). The protocol used to induce small intestinal inflammation was similar to that previously described in the rat (Kraneveld *et al.*, 1993). To investigate the role of sensory nerves, the influence of capsaicin-induced depletion of sensory neuropeptides on the DTH-induced changes of small intestinal vascular permeability was examined. Furthermore, the importance of tachykinins in the development of the effector phase of the small intestinal DTH reaction was studied by use of selective tachykinin receptor antagonists.

Methods

Male Balb/c mice (6–8 week, Central Animal Laboratory (GDL) Utrecht University, Utrecht, The Netherlands) were used in all experiments. The experiments were approved by the Animal Care Committee, Utrecht University.

Induction of the DTH response in the small intestine

Dinitrofluorobenzene (DNFB, 5 mg ml^{-1}, 100 µl) or vehicle (acetone:olive oil = 4:1) was applied on the surface of the skin of the shaven thorax and feet of anaesthetized mice (sodium pentobarbitone, 30 mg kg^{-1} i.p., n = 5/6) on 2 consecutive days. On day 5 the animals were challenged intranasally with dinitrobenzene sulphonic acid (DNBS, 6 mg ml^{-1}, 50 µl). In preliminary investigations, it was observed that Evans blue dye administered intranasally was found in substantial amounts in the gastrointestinal tract of mice, demonstrating that drugs administered intranasally gain access to the intestine.

Small intestinal vascular permeability changes induced by DTH reaction

To assess vascular leakage, Evans blue dye (EB, 12.5 mg ml^{-1} in sterile saline, 50 µl) was administered intravenously to anaesthetized mice (i.p. sodium pentobarbitone 30 mg kg^{-1}) 2 h before the animals were killed. Evans blue dye binds to plasma proteins and can be used as a marker of plasma protein extravasation (Udaka *et al.*, 1970). Shortly before the mice were killed heparin was administered i.v. (10 u ml^{-1} blood) and a blood sample was taken. After the animal had been killed the intestines were vascularly perfused with warm saline (5 ml) to eliminate the excess of vascular Evans blue. The Evans blue dye was extracted from tissue-segments of the small intestine overnight in formamide at 40°C and the amount of dye in extracts and plasma samples was determined with a spectrophotometer at 620 nm (Rogers *et al.*, 1989). The amount of Evans blue dye was expressed as ng per mg small intestinal dry weight and corrected for the plasma EB concentration. Intestinal vascular leakage was examined 0–2, 22–24 and 46–48 h after the DNBS challenge. The following groups were studied: DNFB/DNBS: DNFB sensitization and DNBS challenge and control DNBS: sham sensitization and DNBS challenge. In addition, mice which were DNFB-sensitized and sham-challenged (DNFB control) and mice which were sham-sensitized and sham-challenged (naive) were also studied.

Effect of depletion of capsaicin-sensitive sensory nerves on DTH-induced changes in vascular permeability

Capsaicin was used to deplete neuropeptides from unmyelinated sensory C fibres. The mice were anaesthetized with sodium pentobarbitone, 30 mg kg^{-1}, and received s.c. injections of capsaicin (25 mg kg^{-1}) on 2 consecutive days. Controls were treated with vehicle (alcohol:Tween 80:saline = 2:1:7) alone. The mice were used 2 weeks after pretreatment with capsaicin. Table 1 shows the groups which were examined to investigate the role of capsaicin-sensitive sensory nerves in DTH-induced changes in small intestinal vascular permeability 2, 24 and 48 h after the challenge.

Table 1 Capsaicin pretreatment of mice

Pretreatment	n	*Sensitization*	*Challenge*
Vehicle	5	DNFB	DNBS
Vehicle	6	Vehicle	DNBS
Capsaicin	5	DNFB	DNBS
Capsaicin	5	Vehicle	DNBS

Pretreatment with capsaicin: 25 mg kg^{-1} s.c. on 2 consecutive days 2 weeks before the sensitization. DNFB: dinitrofluorobenzene; DNBS: dinitrobenzene sulphonic acid; vehicle:alcohol:Tween 80:saline = 2:1:7.

To assess the degree of neuropeptide depletion, we studied a neurogenic inflammatory cutaneous reaction in separate groups of capsaicin- or vehicle-pretreated mice. Neurogenic inflammation was induced by topical administration of 40 µl capsaicin solution (1 mg ml^{-1}) onto one ear. The inflammatory oedema formation in the skin was assessed by punching out ear discs (diameter: 8 mm) and differences between the weight of the capsaicin-challenged ear and the non-challenged ear were expressed in mg.

Direct effect of tachykinins, substance P and neurokinin A on small intestinal vascular permeability in naive mice

The tachykinins, substance P and neurokinin A, were given intravenously to non-sensitized and non-challenged 'naive' animals (1.5×10^{-11}–5×10^{-10} mol per mouse in sterile saline, n = 5/6 mice). In addition the effects of three different neurokinin receptor antagonists were studied on the basal small intestinal vascular leakage and on the substance P-induced change in small intestinal permeability. The following neurokinin receptor antagonists were tested: the selective NK_1 receptor antagonist FK888 (10^{-9} mol per mouse); the selective NK_1 receptor antagonist RP67580 or its inactive enantiomer RP68651 (10^{-9} mol per mouse); the nonselective NK_1/NK_2 receptor antagonist FK224 (5×10^{-9} mol per mouse). Each neurokinin receptor antagonist was injected i.v. simultaneously with substance P (5×10^{-11} mol per mouse). In addition, RP67580 was also tested on the NKA (5×10^{-11} mol per mouse, i.v.) and PAF (2×10^{-11} mol per mouse, i.v.)-induced increase in vascular leakage. After 1 h the small intestinal vascular leakage was assessed with Evans blue dye as described above.

Effect of NK_1 receptor antagonist, RP67580 treatment on the DTH-induced changes in small intestinal vascular permeability

In further experiments, the effect of the NK_1 receptor antagonist, RP67580, on the DTH-induced changes of vascular permeability in the small intestine was studied 24 h after the DNBS challenge in DNFB-sensitized or control animals. RP67580 (10^{-9} mol per mouse per injection, n = 5/6 mice per treatment) was administered intravenously using three treatment regimes: (i) immediately before and 1 h after the challenge; (ii) 22 and 23 h after the challenge and (iii) immediately before and 1 h, 22 and 23 h after the challenge. We also studied the effect of the inactive enantiomer RP68651 (10^{-9} mol per mouse; treatment regime (iii)) on the DTH-induced increase in vascular leakage 24 h after the challenge.

Materials

DNFB, substance P and neurokinin A were purchased from Sigma (St. Louis, MO, U.S.A.). DNBS was obtained from Eastman Kodak Co. (Rochester New York, U.S.A.). Evans blue dye, capsaicin and Tween 80 were obtained from Fluka

Chemika (Buchs, Switzerland). Sodium pentobarbitone (Nembutal) was from Sanofi (France). The nonpeptide NK_1 receptor antagonist, RP67580, (3aR, 7aR)-7,7-diphenyl-2-(1-imino-2-(2-methoxyphenyl)ethyl)perhydroisoindol-4-one, and its inactive enantiomer RP68651, (3aS, 7aS)-7,7-diphenyl-2-(1-imino-2-(2-methoxyphenyl)ethyl)perhydroisoindol-4-one, were gifts from Rhône-Poulenc (Rorer, France). The dipeptide NK_1 receptor antagonist, FK888, N^2[(4R)-4-hydroxy-1-(1-methyl-1H-indol-3-yl)carbonyl-L-propyl]-*N*-methyl-*N*-phenyl methyl-3-(2-naphthyl)L-alaninamide, and the peptide NK_1 and NK_2 receptor antagonist, FK224, N-[N^2-[N-[N-[N-[2,3-didehydro-*N*-methyl-*N*-[N-[3-(2-penthylphenyl)-propionyl]-L-threonyl]tyrosyl]-L-leucynyl]-D-phenylalanyl]-L-*allo*-threonyl]-L-asparaginyl]-L-serine-*v*-lactone, were gifts from Fujisawa Pharmaceuticals Co (Osaka, Japan). RP67580, RP68651, FK224 and FK888 were dissolved in ethanol and stored in stock solutions of 10^{-3} M. Further dilutions were made in sterile saline.

Data analysis

The results are presented as mean ± s.e.mean. The DTH-induced changes in small intestinal permeability were analysed by analysis of variance (ANOVA), and the statistical significance of differences between means was determined with the Newman-Keuls test. The assessment of neuropeptide depletion in the skin, the effects of substance P, neurokinin A and the effects of the neurokinin receptor antagonists on small intestinal vascular leakage were evaluated statistically with Student's *t* test (unpaired). All *P* values <0.05 were considered to reflect a statistically significant difference.

Results

DTH-induced changes in small intestinal vascular permeability in the mouse

Basal small intestinal vasopermeability in naive mice was 46.4 ± 3.6 ng EB mg^{-1} dry weight. Figure 1a shows that 0–2 h after the DNBS challenge there were no significant differences in small intestinal vascular leakage values between DNFB/DNBS mice and the control group (control/DNBS). The DNBS challenge alone induced a significant increase in vascular leakage 24 and 48 h after the challenge when compared to basal level (Figure 1a). However, a more profound significant increase in vascular permeability was found 22–24 h after DNBS challenge in DNFB-sensitized mice (DNFB/DNBS: 207.8 ± 25.1 ng EB mg^{-1} dry weight compared to the control/DNBS: 108.2 ± 8.6 ng EB mg^{-1} dry weight, $P<0.01$). It was noted that the DTH-induced vascular leakage response had returned to control levels 46–48 h after the challenge. In addition, the vascular permeability values of mice, which were DNFB-sensitized and sham-challenged, did not differ significantly from the values of non-sensitized and non-challenged mice (data not shown).

Effect of depletion of capsaicin-sensitive sensory nerves on DTH-induced changes in vascular permeability

As demonstrated in Table 2, topical administration of capsaicin resulted in a significant increase in ear weight indicating neurogenic inflammation in the skin. Pretreatment with capsaicin 2 weeks before the topical capsaicin challenge prevented this neurogenic inflammation. Therefore, it was concluded that the pretreatment with capsaicin on 2 consecutive days resulted in a depletion of neuropeptides in the mouse after 2 weeks.

This protocol was used to investigate further the role of capsaicin-sensitive nerves in the DTH-induced small intestinal inflammation. Pretreatment with capsaicin completely inhibited the profound increase in small intestinal vascular permeability observed 22–24 h after the DNBS challenge in DNFB-sensitized mice (Figure 1b). In addition, depletion of capsaicin-sensitive sensory nerves also abolished the increased vascular permeability caused by DNBS challenge in non-sensitized mice 24 and 48 h after the challenge (Figure 1b).

Direct effect of tachykinins substance P and neurokinin A on small intestinal vascular permeability in naive mice

The direct effects of intravenously injected substance P (SP) and neurokinin A (NKA) were investigated on small intestinal vascular leakage in naive mice. Both tachykinins induced an increase in vascular permeability as reflected by an increase in Evans blue tissue accumulation (Table 3). This substance P response was dose-dependent (vehicle: 64.6 ± 4.0; 1.5×10^{-11} mol per mouse: 92.7 ± 8.1; 5×10^{-11} mol per

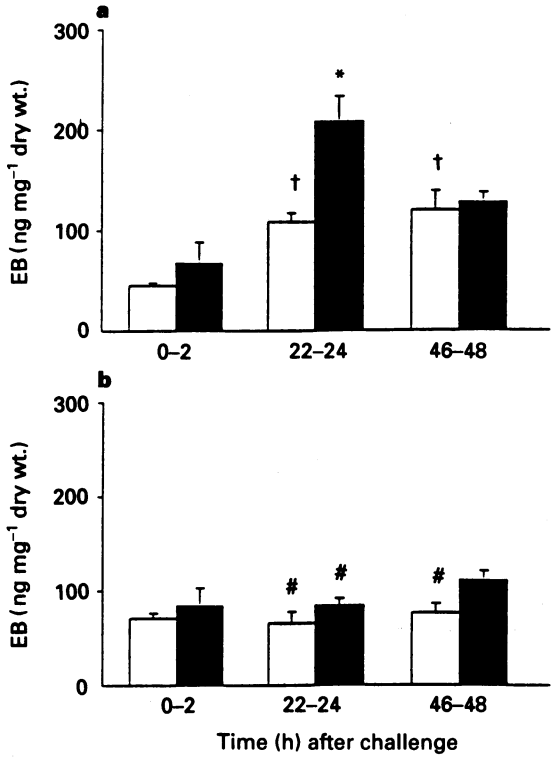

Figure 1 DTH reaction in the small intestine of vehicle-(a) and capsaicin-(b) pretreated mice 2, 24 and 48 h after challenge. Pretreatment with capsaicin, 25 mg kg^{-1}, s.c. or saline on 2 consecutive days was performed 2 weeks before the sensitization. Changes in small intestinal vascular leakage after intranasal administration of DNBS in DNFB-sensitized mice (DNFB/DNBS: solid column) and control mice (control/DNBS: open column) are expressed as Evans blue tissue accumulation (ng mg^{-1} dry weight) for 5 or 6 mice per group and shown as mean ± s.e.mean. *$P<0.05$ compared to control groups; †$P<0.05$ compared to basal vascular leakage (46.4 ± 3.6 ng EB mg^{-1} dry weight) and #$P<0.05$ compared to vehicle pretreatment, ANOVA, Newman-Keuls test.

Table 2 Effect of capsaicin-induced neuropeptide depletion on neurogenic inflammatory responses to topical capsaicin (1 mg ml^{-1}, 40 µl) applied to the mouse ear

Pretreatment	n	*Ear-disk weight difference* (mg)
Vehicle	6	7.28 ± 0.87
Capsaicin	9	0.43 ± 0.28*

Mice were pretreated with capsaicin (25 mg kg^{-1}, s.c.) on 2 consecutive days 2 weeks prior to these tests. The results are expressed as mean ± s.e.mean for *n* mice. *$P<0.05$ compared with vehicle pretreatment, Student's *t* test.

Table 3 Effects of the NK_1/NK_2 receptor antagonist, FK224, the NK_1 receptor antagonist, FK888, and the NK_1 receptor antagonist, RP67580, and its inactive enantiomer, RP68651, on basal small intestinal vascular leakage and on substance P-induced increase of small intestinal vascular leakage (RP67580 was also tested on NKA- and PAF-induced increase in vascular leakage)

	Evans blue tissue accumulation (ng mg^{-1} dry weight)			
Antagonist	*Saline*	(n)	*Substance P*	(n)
Vehicle	65.1 ± 2.4	(11)	122.9 ± 10.8*	(11)
FK224	100.1 ± 5.2#	(6)	100.9 ± 6.0#	(6)
FK888	71.1 ± 4.7	(6)	94.9 ± 6.3*,#	(6)
RP67580	73.3 ± 6.3	(10)	77.0 ± 5.7#	(10)
RP68651	69.5 ± 8.0	(5)	134.7 ± 16.7*	(5)
	Saline	(n)	*NKA*	(n)
Vehicle	73.8 ± 7.3	(4)	118.8 ± 7.3*	(4)
RP67580	66.8 ± 5.8	(4)	70.4 ± 3.5#	(4)
	Saline	(n)	*PAF*	(n)
Vehicle	61.9 ± 2.4	(5)	115.6 ± 8.2*	(5)
RP67580	62.0 ± 2.9	(5)	116.8 ± 5.7*	(5)

The mice were injected intravenously. Doses: substance P and NKA: 5×10^{-11} mol per mouse; PAF: 2×10^{-11} mol per mouse; FK224: 5×10^{-9} mol per mouse, FK888, RP67580 and RP68651: 10^{-9} mol per mouse. The results are expressed as mean ± s.e.mean Evans blue tissue accumulation (ng mg^{-1} dry weight) for n mice per group. $*P<0.05$ compared to saline-treated mice and $\#P<0.05$ compared to vehicle pretreatment, Student's t test.

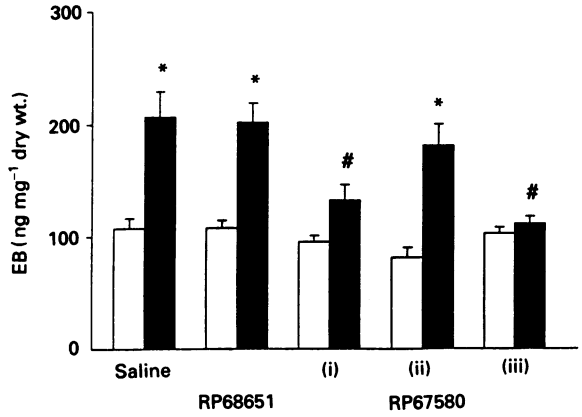

Figure 2 The effects of treatment with the NK_1 receptor antagonist, RP67580 (10^{-9} mol per mouse per i.v. injection) and with the inactive enantiomer, RP68651 (10^{-9} mol per mouse per i.v. injection) on the DNFB/DNBS-induced DTH reaction in the small intestine 22–24 h after DNBS challenge in DNFB- (solid columns) or vehicle- (open columns) sensitized mice. Three regimens of treatments with RP67580 were applied: (i) immediately before and 1 h after the challenge; (ii) 22 and 23 h after the challenge and (iii) immediately before and 1 h, 22 and 23 h after the challenge. RP68651 was administered according to treatment regimen (iii). The vascular leakage responses are expressed as Evans blue tissue accumulation (ng mg^{-1} dry weight) for 5 or 6 mice per group. Means ± s.e.mean are given. $*P<0.05$ compared to control group and $\#P<0.05$ compared to saline/DTH group, ANOVA, Newman-Keuls test.

mouse: 122.9 ± 10.8; 50×10^{-11} mol per mouse: 109.9 × 8.2 ng EB mg^{-1} dry weight, $P<0.05$ compared to vehicle-treatment for all substance P doses, Student's t test) and maximal at a dose of 5×10^{-11} mol per mouse. The increase in small intestinal vascular leakage induced by the tachykinins in naive mice, provided us with a system to test several NK_1 and NK_1/NK_2 receptor antagonists. Table 3 shows that the specific NK_1 receptor antagonist, RP67580 (10^{-9} mol per mouse), reduced the substance P- (5×10^{-11} mol per mouse) induced vascular leakage most profoundly by 94%, whereas its inactive enantiomer RP68651 (10^{-9} mol per mouse) did not influence the substance P response. RP67580 also inhibited the NKA vascular response by 91%. Moreover, RP67580 did not have an effect on the PAF-induced increase in vascular leakage. FK888 (10^{-9} mol per mouse), another specific NK_1 receptor antagonist, reduced the substance P-induced vascular leakage in the small intestine by 57%. The two NK_1 receptor antagonists, FK888 and RP67580, did not affect basal vascular leakage in the small intestine. The nonspecific NK_1/NK_2 receptor antagonist FK224 (5×10^{-9} mol per mouse) induced a small but nonsignificant inhibition of the substance P-induced vascular permeability change. However, intravenous injection of FK224 alone resulted in a significant increase in vascular leakage compared to control. In further experiments RP67580 was used to investigate the role for tachykinins in the DTH response in the mouse small intestine.

Effect of NK_1 receptor antagonist RP67580 treatment on the DTH-induced changes in small intestinal vascular permeability

Figure 2 shows that intravenous administration of RP67580 (10^{-9} mol per mouse per injection) significantly reduced the DTH-induced vascular response by 63% when given immediately before and 1 h after the DNBS challenge (treatment regime (i)). Administration of RP67580 at 22 and 23 h after the challenge (treatment regime (ii)) did not have a significant effect on the DTH response. Treatment with RP67580 immediately before and 1, 22 and 23 h after the challenge (treatment regime (iii)) reduced the DTH-induced increase in vascular permeability by 91%. In contrast, treatment with the inactive enantiomer RP68651 (treatment regime (iii)) did not affect the DTH response. The compounds, RP67580 and RP68651, had no influence on small intestinal vascular leakage in control/DNBS mice.

Discussion

The major finding in the present study is that depletion of sensory neuropeptides and blockade of the NK_1 receptor inhibit DTH-induced changes of small intestinal vasopermeability in the mouse. In addition, we have shown that the tachykinins, substance P and neurokinin A, are able to induce an increase in small intestinal vascular leakage and that this leakage could be blocked by NK_1 receptor antagonists. These results demonstrate that the sensory neuropeptides, especially tachykinins, are involved in the development of DTH-induced increase in plasma extravasation in the small intestine of the mouse.

The DTH response in the small intestine of the mouse was characterized by an increase in vascular permeability 24 h after challenge. The time course of the DTH reaction was different in rats, which showed a significant DNFB/DNBS-induced increase of vascular leakage 48 h after the challenge (Kraneveld *et al.*, 1993). This could be the result of species differences, or difference in dose of the DNBS challenge or in its pharmacokinetics.

In this study it was demonstrated that depletion of sensory neuropeptides by pretreatment with capsaicin abolished the DTH-induced increase in vascular permeability in the small intestine of the mouse. Interestingly, capsaicin pretreatment also inhibited the non-specific DNBS-induced increase in vascular permeability in non-sensitized animals. This non-immunological increase in small intestinal vascular leakage could be the result of an irritant effect of the hapten in which sensory neuropeptides play a role. It has been reported that

single intrarectal installation of the hapten, trinitrobenzene sulphonic acid (TNBS), produces a damaged colon in unsensitized rats; however, in these studies considerably higher doses of the hapten were used (Rachmilewitz *et al.*, 1989; Yamaki *et al.*, 1992).

Several studies have shown that the *in vivo* manipulation of the nervous system resulted in altered immune responses, including those involved in DTH reactions (Macris *et al.*, 1970; Jankovic & Isakovic, 1973; Maric & Jankovic, 1987; Josefsson *et al.*, 1991). More recently, in an immediate-type hypersensitivity reaction in the small intestine, it was shown that tetrodotoxin-induced neuronal blockade inhibited the antigen-induced increase in intestinal permeability in sensitized rats (Crowe *et al.*, 1993). Few reports have described the contribution of peptidergic neurones in the expression of DTH reactions. In the skin, Giolomi & Tigelaar (1990) have shown that early ear swelling induced by the hapten DNFB in mice, 2 h after the challenge, was markedly reduced by capsaicin pretreatment. In the intestinal tract, Maggi *et al.* (1987) showed that depletion of sensory neuropeptides from capsaicin-sensitive nerves significantly increased the degree and incidence of ulceration of the rat duodenum. However, they also reported that in the same intestinal segment, capsaicin-induced increase in vascular permeability was absent in depleted rats.

To characterize further the involvement of sensory neuropeptides in DTH-induced intestinal inflammation, the role of tachykinins in the effector phase of the DTH reaction was investigated. Firstly, substance P and neurokinin A were shown to induce an increase in small intestinal vascular leakage in naive mice. However, the DTH-induced increase in vasopermeability was more profound than the tachykinin-induced increase in vascular leakage. The NK_1 receptor antagonist, RP67580, was most effective in reducing the substance P-induced increase in small intestinal vascular leakage when compared with FK224 and FK888. Since the inactive enantiomer RP68651 did not influence the substance P response and RP67580 did not affect PAF-induced increase in small intestinal vascular leakage, it can be concluded that substance P acts selectively via the NK_1 receptor. In addition, RP67580 significantly inhibited the NKA vascular response in the mouse small intestine. The effects of tachykinins on vascular permeability are to a large extent mediated by NK_1 receptors on endothelial cells (Ohkubo & Nakanishi, 1989). Autoradiographically, it was demonstrated that binding sites for tachykinins exist on the vasculature of the digestive tract. Submucosal arterioles and venules in the intestine were shown to express only NK_1 receptors, whilst NK_2 binding sites were totally absent (Gates *et al.*, 1988; Holzer, 1992). Recently, Beaujouan *et al.* (1993) have reported a higher potency of RP67580 in the mouse and the rat compared with other NK_1 receptor antagonists. Our results suggest that NK_1 receptors are involved in the substance P- and NKA-induced changes in vascular leakage in the mouse small intestine.

RP67580 was used to investigate the role of NK_1 receptors in the DNFB-induced small intestinal DTH response in the mouse. Treatment with RP67580 immediately before and 1 h after the DNBS challenge in DNFB-sensitized mice resulted in a significant reduction of small intestinal vascular leakage at 24 h. In contrast, administration of RP67580 at 22 and 23 h after the challenge did not have a significant effect. Since the inactive enantiomer RP68651 did not affect the DTH response, it can be concluded that the NK_1 receptor plays a role in the initiation of the DTH-induced vascular responses.

It is reasonable to speculate that neuropeptides have an important role in the initiation, maintenance and symptoms associated with inflammatory responses. The gastrointestinal tract is densely innervated by sensory nerves. Immunohistological studies have demonstrated substance P immunoreactivity in nerves throughout the wall of the gastrointestinal tract (Furness & Costa, 1987; Costa *et al.*, 1987; 1991). In addition, it was demonstrated that receptors for substance P were upregulated on blood vessels and lymphoid follicles in Crohn's disease and ulcerative colitis (Mantyh *et al.*, 1988). In animal models of gastrointestinal inflammation, substance P levels were raised during infection (Swain *et al.*, 1992). Karmeli *et al.* (1991) showed that ethanol-induced gastric mucosal damage is mediated by substance P.

Several possibilities can explain the effects of the NK_1 receptor blockade on the DTH reaction. Firstly, many reports have described the close association between mast cells and sensory nerves within the gastrointestinal tract (Skofitsch *et al.*, 1985; Stead *et al.*, 1987b; Crivellato *et al.*, 1991). The mast cell in the gut wall has been reported to be an important immune effector cell, especially in the initiation of DTH responses. It has been hypothesized that stimulation of mast cells leads to the initiation of a cascade of cellular infiltration to develop a DTH response (Askenase & van Loveren, 1983). In addition, substance P induces mediator release from different mast cell types *in vitro* (Shanahan *et al.*, 1985; Assem *et al.*, 1989). The observation of the release of airway mast cell mediators *in vivo* by substance P and neurokinin A, supports the growing belief that mast cells are under sensory nervous control (Joos & Pauwels, 1993). Furthermore, the existence of NK_1 receptors on mast cells has been reported (Joos *et al.*, 1993). Very recently, it has been demonstrated that intestinal mucosal mast cells are activated 30 min after DNBS challenge of DNFB-sensitized rats (Kraneveld *et al.*, 1994). Depletion of sensory neuropeptides or treatment with a NK_1 receptor antagonist immediately before and 1 h after the challenge could, therefore, inhibit the initiating role of the mast cell in our model of gastrointestinal inflammation. Secondly, neuropeptides have a range of effects on the proliferation, migration and activation of lymphocytes. In general, it seems that the tachykinins, substance P and neurokinin A have predominantly stimulatory activity, whereas calcitonin gene-related peptide, vasoactive intestinal peptide and somatostatin have predominantly inhibitory activities (Stanisz *et al.*, 1986). The T lymphocyte is one of the key inflammatory cells in DTH responses. In addition, receptors for substance P have been found on T lymphocytes, although they were not characterized into NK_1, NK_2 or NK_3 receptors (Croitoru *et al.*, 1990). Thus, inhibition of the stimulatory action of substance P on lymphocytes by NK_1 receptor blockade or neuropeptide depletion, could underly the marked reduction of inflammatory response observed in DTH-induced small intestinal inflammation. Finally, a role of neuropeptides in the increase in vascular leakage in DNFB-sensitized and DNBS-challenged mice can be explained by the direct inflammatory actions of neuropeptides: vasodilatation and plasma extravasation, mediated via NK_1 receptors on endothelial cells. One would expect that NK_1 receptor blockade at the time of the DTH response would have an effect. This phenomenon was not observed.

The relevant biological actions together with the close association between sensory nerves, mast cells and immune cells in the gastrointestinal tract, suggests a possible function for neuropeptides in inflammatory responses. In this study, an important role for sensory nerves and for the NK_1 receptor in the DNFB/DNBS-induced DTH reaction in the small intestine of the mouse is demonstrated. Tachykinins could play an important role in the initiation of DTH-induced vascular leakage either indirectly via mast cells or lymphocytes or directly on endothelial cells.

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