Role of mucosal mast cells in early vascular permeability changes of intestinal DTH reaction in the rat

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Kraneveld, Aletta D., Thea Muis, Andries S. Koster, and Frans P. Nijkamp. Role of mucosal mast cells in early vascular permeability changes of intestinal DTH reaction in the rat. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G832–G839, 1998.—Previously, it was shown that depletion and stabilization of the mucosal mast cell around the time of challenge were very effective in reducing delayed-type hypersensitivity (DTH) reactions in the small intestine of the rat. The role of mucosal mast cells in the early component of intestinal DTH reaction was further investigated in this study. In vivo small intestinal vascular leakage and serum levels of rat mast cell protease II (RMCP II) were determined within 1 h after intra gastric challenge of rats that had been sensitized with dinitrobenzene 5 days before. A separate group of rats was used to study vasopermeability in isolated vascularly perfused small intestine after in vitro challenge. To investigate the effects of mast cell stabilization on the early events of the DTH reaction, doxantrazole was used. The influence of sensory nerves was studied by means of neonatal capsaicin-induced depletion of sensory neuropeptides. Within 1 h after challenge, a significant increase in vascular permeability was found in vivo as well as in vitro. This was associated with a DTH-specific increase in RMCP II in the serum, indicating mucosal mast cell activation. In addition, doxantrazole treatment and capsaicin pretreatment resulted in a significant inhibition of the DTH-induced vascular leakage and an increase in serum RMCP II. These findings are consistent with an important role for mucosal mast cells in early vascular leakage changes of intestinal DTH reactions. In addition, sensory nervous control of mucosal mast cell activation early after challenge is demonstrated.

Small intestinal vascular permeability; capsaicin

CONSIDERABLE EVIDENCE supports a role for mast cells in immunologic inflammatory processes (6). Also, in cell-mediated delayed-type hypersensitivity (DTH) reactions a role for mast cells has been postulated (9, 25). DTH reactions in the gastrointestinal tract have been proposed to represent some of the features prevalent in inflammatory bowel diseases (IBD); ongoing responses have been associated with an increased vascular permeability and enhanced lymphocyte infiltration into the inflamed intestinal tissue (10, 13, 31). Most of the studies investigating the role of mast cells in DTH reactions have been done in the intestine, lung, and skin of Trichinella spiralis-infected mice and picryl chloride-contact-sensitized mice (9, 25–27). It has been suggested that on contact sensitization with picryl chloride or after primary helminth infection, DTH-initiating cells in lymphoid organs are induced to release antigen-specific factors that bind systemically to mast cells (4, 15, 22, 36). On local challenge with the antigen, the armed mast cells are activated to release serotonin. Activation of serotonin receptor on vascular endothelium induces a local increase in vascular permeability that facilitates the entry into the tissue of the classical, lymphokine-producing DTH effector T cells (T_{DTH}) (15, 22, 36). These T_{DTH} cells recruit a perivascular leukocyte infiltrate characteristic of DTH reactions and induce mast cell proliferation. Both the T cell factor-dependent early phase component (0–2 h) and the classical late (24–48 h) delayed component of DTH reactions are expressed by an increase in vasopermeability of challenged cutaneous, lung, and intestinal sites (4, 35, 36).

We previously showed (18) that small intestinal DTH responses to the contact sensitizer 2,4-dinitro-1-fluorobenzene (DNFB) in the rat were characterized by an inflammatory response, intestinal mucosal mast cell activation, and tissue accumulation of mucosal mast cells 48–72 h after local challenge. In addition, pharmacologic manipulation of the mucosal mast cell before and at time of challenge, either by depletion or stabilization, was very effective in reducing the DTH-specific increase in small intestinal vascular leakage and mucosal mast cell degranulation found at 48 h (20). Using mast cell-deficient mice, we demonstrated (21) that mast cells contribute significantly to changes in vascular permeability associated with small intestinal DTH reaction. These results suggested that the mucosal mast cell is an important cell in the initiation of contact sensitizer-induced DTH reactions in the small intestine of the rat.

Sensory neurons densely innervate the gastrointestinal tract and are found in close association with lymphocytes and mucosal mast cells (3, 8, 33, 34). The effects of neuropeptides on immunologic functions involved in DTH reactions are clearly described: directly via lymphocyte proliferation, maturation, and activation and indirectly via activation of mucosal mast cell or via effects on the vascular endothelium (30, 32). Substance P has the ability to activate mucosal mast cells, and substance P-induced inflammatory responses are shown to be mast cell dependent (12, 37). Moreover, we have demonstrated that sensory nerves are involved in the development of dinitrobenzene-induced small intestinal DTH reactions in the mouse (17). The results were consistent with an initiating role of sensory neuropeptides, especially tachykinins, in dinitrobenzene-induced intestinal DTH reactions.

The relevant biological actions together with the close association between mast cells and sensory nerves in the gastrointestinal tract suggest a possible function for mast cell-sensory nerve interaction in the early component of DTH reactions. Taking the above-described studies together, it can be postulated that at the time of challenge the mucosal mast cell is activated...
via sensory neuropeptides. This early phase process is necessary for the development of the late-phase inflammatory reaction (edema formation, tissue damage, and mucosal mast cell activation and accumulation). In the present study, the role of mucosal mast cells in the early phase of the dinitrobenzene-induced DTH reaction in the rat small intestine was examined in detail. First, we investigated whether mucosal mast cells are activated shortly after the challenge. Rat mast cell protease II (RMCP II), a unique protease located within the granules of intestinal mucosal mast cells, was used as a serum marker for mast cell degranulation. Second, we examined whether this mucosal mast cell activation leads to early phase vascular permeability changes, which in turn facilitate the entry into the tissue of classical DTH effector cells. The vascular permeability changes were studied from 0 to 1 h after the challenge both in vivo and in vitro in the isolated vascularly perfused small intestine (19). The effects of the mast cell stabilizer doxantrazole on the early DTH-induced changes in small intestinal vascular permeability and RMCP II serum levels were investigated in vivo. Finally, to investigate whether the DTH-induced mucosal mast cell activation and early phase vascular permeability changes were under sensory nervous control, the influence of capsaicin-sensitive nerves on the early phase events of the small intestinal DTH response were studied. Neonatal pretreatment with capsaicin was used to deplete sensory neuropeptides. The effect of this neuropeptide depletion was examined in vivo on DTH-induced changes in small intestinal vascular permeability and mucosal mast cell activation observed early after the challenge.

METHODS

Male Wistar rats (Utrecht x Wistar Unilever, 200–250 g; Utrecht University, Utrecht, The Netherlands) that had free access to food (Rnh tm-11/10; Hopefarm, Woerden, The Netherlands) and tapwater were used. The experiments were approved by the Animal Care Committee of Utrecht University.

Sensitization and challenge procedure. DNFB (5 mg/ml, 300 µl) or vehicle (acetone/olive oil, 4:1, 300 µl) was applied epicutaneously to the shaven dorsal skin of anesthetized rats (60 mg/kg body wt pentobarbital sodium; Fluka Chemika, Buchs, Switzerland) and tapwater were used. The experiments were performed via cardiac puncture, and the perfusate was taken. The intestines were perfused with warm saline (30 ml) to eliminate the excess of vascular Evans blue. Perfusion was performed via cardiac puncture, and the perfusate was allowed to depart via a cut vein in the hind paw. Accumulated Evans blue dye in the small intestine was extracted overnight using formamide (Merck, Darmstadt, Germany) at 40°C. Evans blue dye (in µg) in extracts and plasma samples was determined using a spectrophotometer (double-beam spectrophotometer UV 150-02, Shimadzu) at 620 nm (38). Over the time interval of 30–60 min after the challenge, the small intestinal vascular leakage was measured. The amount of Evans blue dye was expressed as nanograms of Evans blue per milligrams of small intestinal dry weight and corrected for the plasma concentration (ng Evans blue/mg dry wt). The dry weight of the pieces of small intestine was determined by drying the parts in a stove at 60°C for ~10 days. If no changes in weight occurred over the 2 following days, this weight was taken as the dry weight.

In vitro small intestinal vascular permeability changes. In vitro assessment of vascular permeability was measured in the isolated vascularly perfused small intestine. On day 5 the entire small intestine from DNFB- or sham-sensitized rats was isolated as described previously (19). Briefly, after cannulation of the superior mesenteric artery and the portal vein, the vascular bed was perfused with medium. This medium consisted of a buffered salt solution containing 20% (wt/vol) perfluorotributylamine (FC-43; 3M, Leiden, The Netherlands), 2.46% (wt/vol) poly(propylene glycol); polyethylene glycol (1:4) (Syperonic F-68; Sevra Feinbiochemica, H üidelberg, Germany), 103 mM NaCl, 4.56 mM KCl, 1 mM MgCl₂, 1.9 mM CaCl₂, 25 mM NaHCO₃, 10 mM glucose, 1% (wt/vol) albumin, and 0.6 mM glutamine and was gassed with 95% O₂:5% CO₂. Perfusion through the vascular bed was recirculated at a rate of 5 ml/min. The lumen was perfused with saline at a rate of 0.5 ml/min. The luminal perfusate was a 0.9% NaCl solution. The small intestine was excised and transferred to a tissue bath, which was kept at 37°C. Thirty minutes after the intestine was isolated, the luminal perfusate was changed to

intraperitoneally (1 ml). The doxantrazole treatment was applied 30 min before and at the time of challenge. This procedure was followed to be sure that at the time of challenge a sufficiently high amount of doxantrazole was present in the rats to stabilize mucosal mast cells in the small intestine. The dose and the route of administration were obtained using the method of Perdue and Galli (28). Capsaicin (Fluka Chemika, Buchs, Switzerland) was used to deplete neuropeptides from unmyelinated sensory C fibers. Neonally, rats pretreated with the analgesic agent flunitrazepam (1 mg/kg body wt ip) received subcutaneous injections of capsaicin (50 mg/kg body wt) on 2 consecutive days. Controls were treated with vehicle (alcohol/Tween 80/saline, 2:1:7) alone. The rats were used 6–7 wk after pretreatment with capsaicin (body wt, 200–225 g). The degree of sensory neuropeptide depletion was checked by verifying that in vitro capsaicin-induced relaxations of isolated carbachol-precontracted tracheas of the capsaicin-pretreated rats were reduced to <10% of control values of tracheas of vehicle-pretreated rats.

In vivo small intestinal vascular leakage. To assess vascular leakage in the small intestine in vivo, Evans blue dye (20 mg/kg body wt in sterile saline; Fluka Chemika) was injected intravenously into anesthetized animals (60 mg/kg body wt pentobarbital sodium) 30 min after the challenge. Heparin (1,000 IU/rat; Leo Pharmaceutical Products, Ballerup, Denmark) was administered intravenously 5 min before the rats were killed. At t = 60 min, the animals were killed (with an overdose of pentobarbital sodium) and a blood sample was taken. The intestines were perfused with warm saline (30 ml) to eliminate the excess of vascular Evans blue dye. Perfusion was performed via cardiac puncture, and the perfusate was allowed to depart via a cut vein in the hind paw. Accumulated Evans blue dye in the small intestine was extracted overnight using formamide (Merck, Darmstadt, Germany) at 40°C. Evans blue dye (in µg) in extracts and plasma samples was determined using a spectrophotometer (double-beam spectrophotometer UV 150-02, Shimadzu) at 620 nm (38). Over the time interval of 30–60 min after the challenge, the small intestinal vascular leakage was measured. The amount of Evans blue dye was expressed as nanograms of Evans blue per milligrams of small intestinal dry weight and corrected for the plasma concentration (ng Evans blue/mg dry wt). The dry weight of the pieces of small intestine was determined by drying the parts in a stove at 60°C for ~10 days. If no changes in weight occurred over the 2 following days, this weight was taken as the dry weight.

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a solution of DNBS (1 mg/ml in 0.9% NaCl) for in vitro challenge of the preparation. Four tracer injections were administered (i.e., before DNBS challenge), 15, 30, and 60 min (i.e., after DNBS challenge) after the start of the DNBS infusion. The following tracers were studied: tetramethylrhodamine-conjugated dextran (TMRD3, 3.0 kDa, 0.03 mg/ml), Cascade blue-conjugated dextran (CBD10, 10 kDa, 0.10 mg/ml), Texas red-conjugated dextran (TRD70, 70 kDa, 0.27 mg/ml), and FITC-conjugated Ficoll (FF400, 400 kDa, 0.27 mg/ml) (Molecular Probes, Eugene, OR). All compounds were dissolved in the vascular perfusate. After the perfusion through the vascular bed was changed from recirculating to single pass, a mixture of the four tracers (200 µl) was injected rapidly into the arterial cannula and total venous drainage was collected in a series of 10 tubes (~300 µl each). The venous samples were weighed to determine volume and collection time. After centrifugation (10 min at 15,000 g max) the concentrations of the four tracers in the supernatants were measured fluorometrically in a 96-well plate reader (LS50B; Perkin Elmer, Gouda, The Netherlands) with TMRD3 at 555/580 nm, CBD10 at 400/425 nm, TRD70 at 591/612 nm, and FF400 at 495/520 nm for excitation and emission wavelength, respectively.

Pharmacokinetic analysis, based on statistical moment theory, of the tracer (i) outflow concentration-time curve was used to assess vasopermeability: extraction ratios (Ei), volumes of distribution (Vdi, i), and intrinsic clearance values (Clint, i). This involves analysis of the complete venous outflow concentration-time curve (dilution curve). The outflow concentrations of the tracers were normalized by expressing the concentration in each sample relative to the concentration in the original injectate (Ci). Subsequently, a relative concentration-time outflow curve was obtained for each tracer used.

The first two moments of the relative concentration-time curve of each tracer on a single pass through the isolated vascularly perfused small intestine were determined for the calculation of the area under the concentration-time curve (AUCi) and the mean transit time (ti).

\[
\text{AUC}_i = \int C_i \, dt
\]

\[
t_i = \left(\frac{1}{tC_i \, dt} / \text{AUC}_i\right)
\]

A vascular tracer (vrs) is a tracer that is not eliminated and retained in the intravascular space. Thus, theoretically, the extraction of a vascular tracer is 0. The extraction ratio for diffusible tracers was calculated by

\[
E_i = 1 - \left(\frac{\text{AUC}_i}{\text{AUC}_{vrs}}\right)
\]

where AUCvrs is the area under the concentration-time curve of the vascular tracer.

The intrinsic clearance (in ml/min) according to the parallel tube model is calculated by

\[
\text{Cl}_{\text{int}, i} = -Q \times \ln(1 - E_i)
\]

where Q is the perfusate flow rate.

The volume of distribution of each tracer (Vdi, i) is calculated (in ml) from the intrinsic clearance by

\[
V_{di, i} = \text{Cl}_{\text{int}, i} \times t_i / E_i
\]

All the data are expressed as either a percentage (Ei), in milliliters (Vdi, i), or in milliliters per minute (Clint,i) per small intestine.

Measurement of RMCP II. The sera were collected via orbital puncture in the anesthetized rats (sham/DNBS and DNFB/DNBS) 15, 30, and 60 min after the challenge, and the samples were snap frozen in liquid nitrogen and stored at –80°C. From a separate group of nonsensitized and nonchallenged (naive) rats, serum samples were collected at t = 0, 15, 30, and 60 min. The RMCP II ELISA (Moredun Animal Health, Edinburgh, UK) was used for measurements of RMCP II in the serum of the groups of rats described above. The results were expressed as nanograms of RMCP II per milliliter of serum.

Data analysis. Results are presented as means ± SE. Data were analyzed by ANOVA, and the statistical significance of difference between means was determined using the Newman-Keuls test. P < 0.05 was considered to reflect a statistically significant difference.

RESULTS

In vivo small intestinal vascular permeability changes. The isolated fluorocarbon vascularly perfused small intestine, a preparation with physiological viability, was used to measure microvascular permeability changes from 15 to 60 min after in vitro DNBS challenge in detail using tracer molecules of different sizes (1.4- to 6-nm radius). FF400 (vrs) was used as the vascular tracer (Clint,vrs = 0 ml/min). The volume of distribution of FF400 (Vdi,vrs = 1.45 ± 0.05 ml) reflects the volume of the mesenteric vasculature between injection and sampling sites. Under control conditions (before in vitro DNBS challenge), the volume of distribution and the intrinsic clearance value of the diffusible tracer TRD70, a molecule that resembles plasma albumin in size, did not differ significantly from the vascular tracer.

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lar tracer. When the molecular radii of the tracers (CBD10 > TMRD3) decrease, an increase in volumes of distribution and intrinsic clearance values was observed. Under control conditions, no significantly different permeability values were found for CBD10 and TMRD3 (Table 1 and Fig. 3). Figure 3A and Table 1 show that in vitro DNBS challenge in sham-sensitized intestinal preparations did not significantly change the vascular permeability characteristics of all the tracers studied. In addition, DNBS challenge in isolated vascularly perfused small intestines of DNFB-sensitized rats did not induce changes of the volume of distribution of the vascular tracer FF400. However, intraluminal DNBS challenge in DNFB-sensitized preparations after 30–60 min significantly increased the volumes of distribution of the diffusible tracers TRD70, CBD10, and TMRD3 (Table 1). The volumes of distribution of CBD10 and TMRD3 (V_{CBD10} vs. V_{TMRD3} 3.07 ± 0.23 vs. 3.13 ± 0.20 ml, respectively) were two times the vascular volume (V_{vess} = 1.59 ± 0.03 ml) 30 min after the DNBS challenge. Figure 3B shows that 15 min after the DNBS challenge the vascular permeability for all diffusible tracers started to rise. The increase in vascular permeability peaked 30–60 min after the challenge. At t = 15 min, the sensitized and challenged mesenteric vasculature was most permeable for the smallest tracer, TMRD3 (Fig. 3B). However, 30–60 min after the DNBS challenge the clearance values for CBD10 and TMRD3 were not statistically different from each other (Fig. 3B). The increase in permeability is seen earlier for the smaller molecules but is more extensive for the larger tracer (TRD70). Intraluminal DNFB challenge did not have an effect on the basal vascular pressure (~90 mmHg) in either sham- or DNFB-sensitized intestinal preparations (data not shown). These results clearly demonstrate that vascular permeability changes early after mast cell activation can be detected using tracer molecules of varying sizes. RMCP II release in serum. RMCP II was determined in serum samples from naive rats as well as from sham- or DNFB-sensitized and DNBS-challenged rats. Basal RMCP II level in the serum of naive rats was ~100 ng RMCP II/ml serum. No differences were observed in RMCP II basal levels when the samples were obtained at t = 15, 30, and 60 min. Figure 4 shows that DNBS challenge alone significantly enhanced the RMCP II levels 15 and 30 min after the challenge. However, in DNFB-sensitized and DNBS-challenged rats, a more profound fourfold increase in RMCP II serum levels was found, which was significantly different from levels of sham-sensitized and DNBS-challenged rats. This elevation of serum RMCP II returned to basal levels 120 min after the DNBS challenge (Fig. 4).

Effect of doxantrazole treatment on small intestinal DTH reaction. Mast cell stabilization induced by doxantrazole injection 30 min before and at time of challenge significantly inhibited the DTH-induced increase in small intestinal vascular permeability 30–60 min after the challenge (Fig. 1). In addition, analysis of RMCP II

Table 1. Extraction ratios and volumes of distribution of tracers in the rat isolated vascularly perfused small intestine

<table>
<thead>
<tr>
<th>Tracer (i)</th>
<th>Radius, nm</th>
<th>Ei, %</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>Vd,i, ml</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
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<tbody>
<tr>
<td>Sham-sensitized rats</td>
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<tr>
<td>FF400</td>
<td>20.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.53 ± 0.03</td>
<td>1.39 ± 0.04</td>
<td>1.43 ± 0.08</td>
<td>1.44 ± 0.04</td>
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<tr>
<td>TRD70</td>
<td>6.0</td>
<td>5 ± 3</td>
<td>3 ± 2</td>
<td>4 ± 2</td>
<td>3 ± 3</td>
<td>1.64 ± 0.06</td>
<td>1.65 ± 0.13</td>
<td>1.74 ± 0.13</td>
<td>1.60 ± 0.12</td>
<td></td>
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<tr>
<td>CBD10</td>
<td>2.2</td>
<td>24 ± 3</td>
<td>26 ± 2</td>
<td>27 ± 3</td>
<td>24 ± 5</td>
<td>2.17 ± 0.11</td>
<td>2.13 ± 0.08</td>
<td>2.13 ± 0.18</td>
<td>2.22 ± 0.12</td>
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<tr>
<td>TMRD3</td>
<td>1.4</td>
<td>23 ± 5</td>
<td>28 ± 4</td>
<td>32 ± 4</td>
<td>28 ± 3</td>
<td>2.25 ± 0.06</td>
<td>2.32 ± 0.09</td>
<td>2.70 ± 0.13</td>
<td>2.35 ± 0.10</td>
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<tr>
<td>DNFB-sensitized rats</td>
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<tr>
<td>FF400</td>
<td>20.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.56 ± 0.03</td>
<td>1.48 ± 0.06</td>
<td>1.59 ± 0.03</td>
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<tr>
<td>TRD70</td>
<td>6.0</td>
<td>3 ± 3</td>
<td>10 ± 2</td>
<td>22 ± 4</td>
<td>16 ± 5</td>
<td>1.57 ± 0.06</td>
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<td>2.08 ± 0.06*</td>
<td>1.94 ± 0.16*</td>
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<tr>
<td>CBD10</td>
<td>2.2</td>
<td>17 ± 4</td>
<td>24 ± 2</td>
<td>39 ± 3*</td>
<td>38 ± 6</td>
<td>2.04 ± 0.14</td>
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<td>3.07 ± 0.23*</td>
<td>2.70 ± 0.17*</td>
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<tr>
<td>TMRD3</td>
<td>1.4</td>
<td>20 ± 5</td>
<td>29 ± 5</td>
<td>38 ± 3</td>
<td>36 ± 6</td>
<td>2.27 ± 0.17</td>
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<td>3.13 ± 0.20*</td>
<td>2.98 ± 0.32*</td>
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Results are means ± SE for n = 4 rats/group. Radius, Stokes-Einstein molecular radius; Ei, extraction ratio; Vd,i, volume of distribution. DFNB, 2,4-dinitro-1-fluorobenzene. FF400, FITC-conjugated Ficoll; TRD70, Texas red-conjugated dextran; CBD10, cascade blue-conjugated dextran; TMRD3, tetramethylrhodamine-conjugated dextran. 0 min, Before dinitrobenzene sulfonic acid (DNBS) challenge 15–60 min, Time after DNBS challenge. *P < 0.05 compared with sham-sensitized rats (ANOVA).
levels in the serum of DTH rats after doxantrazole pretreatment showed that this compound was effective in reducing the DTH-induced elevation of serum RMCP II (Fig. 5).

Effect of sensory neuropeptide depletion on small intestinal DTH reaction. Figure 2 shows that neuropeptide depletion completely inhibited the increase in small intestinal vascular permeability observed 30–60 min after challenge in DNFB-sensitized rats. No effects of capsaicin pretreatment were found on basal vascular leakage. Furthermore, depletion of neuropeptides completely abolished the DTH-induced increase in serum RMCP II levels observed 30 min after challenge (Fig. 6).

DISCUSSION

The aim of this study was to investigate the role of the mucosal mast cell in the early vascular permeability changes of dinitrobenzene-induced DTH reactions in the small intestine of the rat. DTH reactions are local T lymphocyte-dependent immune responses manifested by an inflammation (35). It has been suggested that such a cell-mediated response plays an important role in the pathogenesis of IBD (13).

DTH reactions are characterized by an early (<2 h after the challenge) and a late component (24–48 h after the challenge). It is hypothesized that the early DTH response is induced by antigen-specific factors released by T lymphocytes within 1–2 days of sensitization, which arm mast cells systemically (26). On local challenge, the T cell factor-armed mast cells release...
There are several lines of evidence that favor a role for the mast cell in the early phase of DTH reactions. First, the reactions are elicited preferentially at sites enriched in mast cells, such as the intestinal tract, lung, buccal mucosa, and skin (1, 9, 25). The release of the mast cell mediator serotonin during contact sensitization in the skin, early after the challenge, has been reported (14, 16, 35). This vasoactive amine can act locally by increasing vascular permeability and inducing vasodilation, thereby facilitating cellular infiltration (2, 4). In addition, corticosteroid-induced mucosal mast cell depletion and treatment with mast cell stabilizers or serotonin antagonists suppressed DTH reaction in the skin and small intestine of the mouse and the rat (2, 4, 14). Defective DTH responses have been observed in strains of mast cell-deficient mice (4, 23). However, no reports have described direct assessment of mast cell activation during the early events of DTH reactions in the small intestine. In this study, we have monitored in vivo mucosal mast cell activation up to 120 min after the challenge by measurement of RMCP II. RMCP II is a protease specific for intestinal mucosal mast cells that appears in the serum of rats after mast cell degranulation (24). Our results demonstrate that mucosal mast cells are activated 15–30 min after DNBS challenge in DNFB-sensitized rats. This mucosal mast cell activation slightly preceded the DTH-induced small intestinal vascular leakage changes found in vivo 30–60 min after the challenge. It even suggests that the DNBS challenge alone has a direct irritating effect on intestinal mucosal mast cells, because of the small rise in RMCP II found in sham-sensitized animals. This DNBS-induced mucosal mast cell activation was not accompanied by changes in vascular permeability in the small intestine after only DNBS challenge. It can be concluded that a more profound mucosal mast cell activation is necessary to result in an increase in vascular permeability.

To further investigate the role of the mucosal mast cell in the early component of small intestinal DTH reactions, rats were pretreated before the challenge with the mast cell stabilizer doxantrazol. This compound is able to prevent antigen-induced histamine release from gut-associated mucosal mast cells and to protect sensitized mucosal mast cells from airway mast cell mediators in vivo by substance P and serotonin.
neurokinin A supports the growing belief that mast cells are under nervous control (12). In this study, it was demonstrated that depletion of neuropeptides from sensory nerves by neonatal pretreatment with capsaicin resulted in significant inhibition of the early vascular leakage DTH response as well as in reduced mucosal mast cell activation. These results confirm the inhibitory effects of neuropeptide depletion and blockade of the neurokinin-1 receptor observed on dinitrobenzene-induced small intestinal DTH reaction in the mouse (17). However, it is unclear whether sensory neuropeptides act directly on the mucosal mast cell in the DTH reaction or indirectly on T cell factor-producing lymphocytes. The tachykinins substance P and neurokinin A predominantly stimulate the proliferation, migration, and activation of lymphocytes (32, 33), whereas vasoactive intestinal peptide, calcitonin gene-related peptide, and somatostatin have inhibitory activities (32). In addition, receptors for tachykinins have been found on lymphocytes (7). Thus neuropeptide depletion could result in a direct inhibition of DTH-induced mucosal mast cell activation or in an inhibition of proliferation and activation of T cell factor-producing lymphocytes, which in turn will lead to less T cell factor-armed mast cells. Neonatal treatment with capsaicin could also affect the number of mucosal mast cells in the small intestine. Recently, Gottwald and colleagues (11) have demonstrated that 3 mo after neonatal capsaicin administration 28% fewer intestinal mucosal mast cells were found in treated rat jejunum compared with littermate controls. In this study, the rats were used at the age of 7–8 wk. It is not likely that at this age neonatal capsaicin pretreatment results in a complete depletion of intestinal mucosal mast cells and that the effects of this treatment found on DTH-induced vascular permeability changes and mucosal mast cell activation are only the result of a lowering of the number of mast cells.

In conclusion, the results of this study are consistent with an important role of the mucosal mast cell in the early vascular events in the dinitrobenzene-induced DTH reaction in the small intestine of the rat. In addition, we have demonstrated an interaction between sensory nerves and mucosal mast cell activation during the initiation phase of the small intestinal DTH response.

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