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Characteristics of histamine-induced leukocyte rolling in the undisturbed microcirculation of the rat mesentery

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1 The main objective of this study was to analyse the role and mode of action of the mast cell mediator histamine in leukocyte-endothelium interactions in small venules *in vivo*. For this purpose, we used a histological approach (combined with intravital microscopy) that allows studies of rapid mediator-induced venular leukocyte accumulation, reflecting leukocyte rolling, in the undisturbed microcirculation of the rat mesentery where rolling is normally absent.

2 We first examined the relative importance of histamine and 5-hydroxytryptamine (5-HT) in acute mast cell-dependent leukocyte recruitment. The mast cell secretagogue compound 48/80 (i.p. for 15 min) induced a marked venular accumulation of polymorphonuclear leukocytes (PMNL) which was almost abolished by combined histamine$_1$ (H_1)- and histamine$_2$ (H_2)-receptor blockade. In contrast, the 5-HT-receptor antagonist methysergide was inactive in this regard. Moreover, exogenous 5-HT was less active than exogenous histamine in evoking venular PMNL accumulation (histamine response dose-dependent; 5-HT response bell shaped). Prostaglandin D_2 did not cause PMNL accumulation.

3 The venular PMNL response to exogenous histamine peaked between 15 min and 1 h, was still significantly elevated at 2 h, and then returned to prechallenge values after 3 h. At all time points, the histamine-induced PMNL accumulation was nearly abolished by i.v. treatment with the polysaccharide fucoidin (which blocks rolling but not firm adhesion *per se*), suggesting that the PMNL response to histamine was due to rolling rather than firm adhesion over the entire 3 h period. At no time point did histamine trigger accumulation of mononuclear leukocytes (MNL).

4 To examine the role of histamine-receptors in the histamine-induced PMNL accumulation (i.e. rolling), the animals were pretreated with diphenhydramine (H_1-receptor antagonist), cimetidine, or ranitidine (H_2-receptor antagonists). Diphenhydramine alone inhibited the venular PMNL response to histamine by 52%, while both H_2-receptor antagonists were completely inactive. However, the combination of cimetidine and diphenhydramine reduced the histamine-induced PMNL rolling by 82%. Furthermore, in contrast to an H_3-receptor agonist, challenge with either the H_1-receptor agonist 2-thiazolylethylamine or two different H_2-receptor agonists (impromidine, dimaprit) was sufficient to provoke significant venular PMNL accumulation.

5 Treatment with the nitric oxide-synthase inhibitor L-NAME did not affect the histamine-induced PMNL rolling. On the other hand, 3 h pretreatment with dexamethasone reduced the PMNL response to histamine by 73%, and flow cytometric analysis showed that the dexamethasone treatment almost completely inhibited binding of soluble P-selectin to rat isolated PMNLs.

6 We conclude that initial leukocyte recruitment after mast cell activation in the rat mesentery is critically dependent on histamine release. The cellular response to histamine was specifically due to PMNL rolling, involved activation of both H_1- and H_2-receptors, and lasted for 2–3 h. Moreover, the histamine-induced PMNL rolling was not dependent on nitric oxide synthesis, but was sensitive to glucocorticoid treatment, possibly via inhibition of expression or function of leukocytic P-selectin ligand(s).

Keywords: 5-Hydroxytryptamine; dexamethasone; flow cytometry; histamine; leukocyte adhesion; leukocyte rolling; microcirculation; nitric oxide; P-selectin; prostaglandin D_2

Introduction

Immediate-type allergic inflammation is triggered by mast cell activation, leading to release of inflammatory mediators such as histamine and different chemotactic factors that cause hyperaemia and increased vascular permeability, followed by gradual infiltration of leukocytes (Zweiman, 1988; Togias *et al*., 1988). The recruitment of leukocytes to areas of inflammation is a multistep process, in which initial slow rolling of leukocytes is a precondition for the subsequent firm adhesion and extravasation (Lawrence & Springer, 1991; Lindbom *et al*., 1992). The rolling adhesive interaction is mediated by the three carbohydrate-binding molecules of the selectin family — constitutive L-selectin on the leukocytes and inducible P- and E-selectin on the endothelium (Carlos & Harlan, 1994). In acute allergic inflammation, P-selectin is of particular interest because this molecule is rapidly (within minutes) translocated from intracellular stores to the endothelial cell surface by histamine (Hattori *et al*., 1989). Moreover, several intravital microscopic studies have documented that topical challenge with histamine or the mast cell secretagogue compound 48/80 can increase leukocyte rolling in small venules of the rat mesentery (Asako *et al*., 1994; Kubes & Kanwar, 1994; Ley, 1994; Thorlacius *et al*., 1994; 1995; Gaboury *et al*., 1995) (shown to be P-selectin-dependent in some studies). Yet, the results of these studies are partly contradictory, and several

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aspects of histamine-induced leukocyte rolling are still unclear. For example, the dose of histamine required to trigger leukocyte rolling, and the relative importance of histamine in mast cell-dependent rolling vary considerably between the different studies. However, the most intriguing discrepancy is that related to the relative importance of different histamine-receptors involved in the rolling response to histamine. Thus, Asako *et al*. (1994) and Kubes & Kanwar (1994) showed that histamine-induced leukocyte rolling was abolished by a histamine$_1$ (H_1)-receptor antagonist, but unaffected by histamine$_2$ (H_2)-receptor antagonism. In contrast, Ley (1994) found that the rolling response to histamine was greatly reduced by blockade of H_2-receptors, while H_1-receptor antagonism was completely inactive. One possible explanation for these discrepant results may be related to variations in the doses and routes of administration of histamine and the different antagonists. Another possibility, due to certain differences in experimental design, is that the 'spontaneous' leukocyte rolling evoked by the preparation required for intravital microscopy may have interfered with the histamine response to varying degrees. As discussed elsewhere in this issue of the Journal (Yamaki *et al*., 1998), the 'spontaneous' preparation-induced rolling associated with standard procedures for intravital microscopy also complicates the analysis of temporal aspects of mediator-induced leukocyte rolling. Moreover, with intravital microscopy, it is difficult to differentiate the microvascular behaviour of granulocytes from that of mononuclear cells, for example after challenge with different mediators.

The aim of this study was to analyse in detail the role and mode of action of histamine in leukocyte-endothelium interactions in small venules *in vivo*, including dose-dependency and duration of action, relative contribution of H_1-, H_2- and H_3-receptors, the role of histamine vs 5-hydroxytryptamine (5-HT) in mast cell-dependent leukocyte recruitment, and potential differences between polymorphonuclear leukocytes (PMNLs) and mononuclear leukocytes (MNLs) in their acute reactivity to histamine and other mast cell-derived mediators. For this purpose, we used a combination of intravital microscopy and histological quantification of intravascular leukocyte concentration, an approach that allows studies of rapid mediator-induced leukocyte rolling in the undisturbed microcirculation of the rat mesentery where there is no baseline rolling (Yamaki *et al*., 1998). Moreover, knowing that histamine triggers vascular production of nitric oxide (NO) (Moncada, 1992) and that glucocorticoids can inhibit histamine-induced oedema formation (Tsurufuji & Ohuchi, 1989), we also used this method to examine potential modulatory actions of NO and dexamethasone on the histamine-induced leukocyte responses.

Methods

Animals and anaesthesia

Adult female Wistar rats (180–210 g) were anaesthetized with equal parts of fluanison/fentanyl (Hypnorm, 10/0.2 mg ml^{-1}) and midazolam (Dormicum, 5 mg ml^{-1}) diluted 1:1 with sterile water (2 ml kg^{-1}, i.m.). The animal experiments were approved by the Regional Ethical Committee for Animal Experimentation.

Intravital microscopy

Intravital microscopy of the rat mesenteric microcirculation was performed as described in detail by Yamaki *et al*. (1998). Very briefly, after laparotomy, the mesentery of anaesthetized, thermocontrolled and tracheotomized rats was mounted under the microscope (Leitz Orthoplan with a x55 Na 0.8 water immersion lens) and superfused with a thermostated bicarbonate-buffered saline solution. The microscopic image was recorded on video tape for subsequent off-line analysis. After preparation, a 20 min equilibration period preceded measurements of blood flow in small mesenteric venules (inner diameter ≈ 30 μm) before and at the end of a 5 min topical challenge with indicated concentrations of histamine, 5-HT or compound 48/80. In order to measure blood flow velocity, fluorescent latex beads with a diameter of 1 μm were injected i.v. (3×10^8 beads per animal) and visualized by fluorescent light epi-illumination (Leitz Ploemopak, filter block I2). Determination of the velocity of at least 15 individual free-flowing beads was performed through frame-by frame analysis of video-recordings. Values for vessel radius (r) and highest detected blood flow velocity (V_{max}) were used to calculate blood flow (Q) from the following relation: $Q = (V_{max}/2)\ \pi r^2$.

Intraperitoneal challenge with mediators

Anaesthetized and thermocontrolled rats were challenged i.p. with histamine, selective histamine receptor agonists, 5-hydroxytryptamine, compound 48/80, prostanglandin D_2 (PGD_2) or human recombinant interleukin-1β (IL-1β) dissolved in 5 ml sterile PBS warmed to 37°C. Control rats received either no treatment or 5 ml 37°C PBS alone. At indicated time points after challenge, the animals were killed by an intracardiac injection of 0.2 ml pentobarbitone (60 mg ml^{-1}). The subsequent rapid cardiac arrest was confirmed by palpation before laparotomy and tissue sampling. Drugs were administered i.v. (histamine and 5-HT antagonists, fucoidin, L-NAME) or i.m. (dexamethasone) at indicated doses and time points. At the time of tissue sampling, arterial blood samples were collected from a tail artery for systemic PMNL and MNL counts.

Sampling of mesenteric tissue, histological procedures, and measurements of intravascular and tissue leukocytes

Samples of intact mesenteric microvascular networks were fixed in paraformaldehyde and glutardialdehyde, stained with Giemsa stain, dried, and mounted on glass slides as described in detail by Yamaki *et al*. (1998). As also described by Yamaki *et al*. (1998), the intravascular PMNL and MNL concentrations in small venules (inner diameter $\approx 20-60$ μm) were calculated by assuming cylindrical vessel geometry. The venular leukocyte content was expressed either as number of leukocytes nl^{-1} vessel volume or, more commonly, as the ratio of venular to systemic leukocyte concentration in order to normalize variations in systemic leukocyte counts (see Yamaki *et al*., 1998 and Results section). Mean values of intravascular leukocyte concentrations in the different groups of animals were based on the mean value of 2–6 vessels in 1–3 mesenteric samples in each animal. An increase in leukocyte concentration in rat mesenteric venules may be used as an index of mediator-induced leukocyte rolling if the relative contribution of rolling and firm leukocyte adhesion is first determined (Yamaki *et al*., 1998). Leukocyte emigration was quantified by counting extravascular PMNLs in 20 defined high-power fields along a randomly selected venule in each preparation, and expressed as number of PMNLs mm^{-2}.

Leukocyte isolation and flow cytometry

EDTA-anticoagulated blood was taken from untreated and dexamethasone treated rats. The blood was mixed 5:3 with

6% dextran 70 (Macrodex) and allowed to sediment for 45 min at room temperature. The leukocyte-rich plasma was collected, washed twice at 150 $\times g$ for 7 min with a large volume of PBS (to remove platelets), and resuspended in HBSS with 0.02% sodium azide at a final concentration of 10^6 cells ml^{-1}. Leukocyte suspensions were incubated with soluble L- or P-selectin (10 $\mu g\ ml^{-1}$) for 30 min at 4°C. Production and characteristics of the soluble selectins (fusion proteins of the extracellular selectin domains and IgG) have been described previously (Watson *et al*., 1990; Foxall *et al*., 1992). L-selectin served as negative control for P-selectin. After the primary incubation, the cells were washed, incubated with fluorescein-conjugated goat $F(ab')_2$-anti-human IgG diluted 1:20 for 30 min at 4°C and washed again. The cells were then exposed to a red cell lyzing solution with 1% formaldehyde (FACS Lysing Solution) and washed once. The same procedure was used for immunofluorescence staining of leukocytic L-selectin, with monoclonal antibody (mAb) HRL2 (hamster-anti-rat-L-selectin, 10 $\mu g\ ml^{-1}$) or isotype-matched hamster IgG (negative control) and fluorescein-conjugated goat $F(ab')_2$-anti-hamster IgG (diluted 1:20). All solutions for incubations and washings of leukocytes from dexamethasone-treated rats contained 1 μM dexamethasone. In some experiments, P-selectin was incubated with leukocytes in the presence of 10 μM EDTA. A FACSort flow cytometer (Becton Dickinson, Mountain View, CA, U.S.A.) was used to analyse (immediately after labelling) PMNL fluorescence intensity (reflecting L- and P-selectin binding and L-selectin expression, respectively) after selective gating, based on forward and side scatter characteristics. With regard to the experiments with soluble selectins, the cytometric settings and the regions of background fluorescence (M1; $\geqslant$97.5% of events for soluble L-selectin) and positive fluorescence events (M2) were the same.

Materials

5-Hydroxytryptamine, cimetidine, compound 48/80, diphenhydramine hydrochloride, histamine dihydrochloride, EDTA, fucoidin, N^{ω}-nitro-L-arginine methylester hydrochloride (L-NAME), pyrilamine maleate and ranitidine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 2-Thiazolylethylamine, dimaprit, impromidine and **R**(α)-methylhistamine were from Smith, Kleine & Beecham Laboratories (Welwyn Garden City, U.K.). Methysergide was from Sandoz (Basel, Switzerland). PGD_2 was from Cascade Biochem. Ltd. (Berkshire, U.K.). Human recombinant IL-1β was from Genzyme Diagnostics (Cambridge, MA, U.S.A.). Dexamethasone (Decadron) was from MSD (Rahway, NJ, U.S.A.). Yellow-green carboxylate-modified 1 μm latex beads (FluoSpheres) were from Molecular Probes (Inc., Eugene, OR, U.S.A.). Soluble L- and P-selectin were from Genentech Inc. (San Fransisco, CA, U.S.A.). Fluorescein-conjugated goat $F(ab')_2$-anti-human IgG was from Binding Site (Birmingham, U.K.), and fluorescein-conjugated goat $F(ab')_2$-anti-hamster IgG from Jackson ImmunoResearch Laboratories (West Grove, PA, U.S.A.). Hamster IgG was from Pharmingen (San Diego, CA, U.S.A.). Macrodex was from Pharmacia (Uppsala, Sweden). FACS lyzing solution was from Becton Dickinson (Mountain View, CA, U.S.A.). Dormicum was from Hoffman-La Roche (Basel, Switzerland). Hypnorm was from Janssen Pharmaceutica (Beerse, Belgium). Mebumal was from Nordvacc Läkemedel AB (Stockholm, Sweden). Giemsa stain was from Karolinska Apoteket (Stockholm, Sweden). Canada balsam was from BDH Laboratory Supplies (Poole, U.K.).

Statistical evaluation

For statistical analysis, two-tailed non-parametric tests were used throughout. Analysis of paired observations was performed by use of the Wilcoxon signed rank test, and independent samples were analysed by the Mann-Whitney rank sum test or Kruskal-Wallis one way analysis of variance on ranks. P values <0.05 were considered significant. Data are expressed as mean$\pm$s.e.mean, and n represents number of animals per group.

Results

Role of histamine and 5-HT in mast cell-dependent venular leukocyte accumulation

As examined in the stained mesenteric whole-mounts, the baseline venular concentrations of MNLs and PMNLs were 20.9 ± 4.3 and 1.5 ± 0.3 cells nl^{-1} vessel volume, respectively, in undisturbed tissue ($n=9$) and 16.2 ± 2.5 and 1.6 ± 0.4 cells nl^{-1}, respectively, after i.p. injection of 37°C PBS ($n=8$), with no significant differences between the two groups. In these two groups of control animals, the venular/systemic leukocyte concentration-ratios were slightly above unity (Figure 1, only data for PBS are shown). On the other hand, after i.p. injection of the mast cell secretagogue compound 48/80 (50 μg in 5 ml PBS) for 15 min, the venular/systemic PMNL concentration-ratio increased to 22 times that in animals receiving only PBS (Figure 1). In contrast, the MNLs were not increased in response to the 15 min stimulation with compound 48/80 (Figure 1).

In order to examine the relative importance of histamine and 5-HT in the mast cell-dependent venular PMNL recruitment, the animals were pretreated i.v. for 10 min with different combinations of mepyramine (H_1-receptor antago-

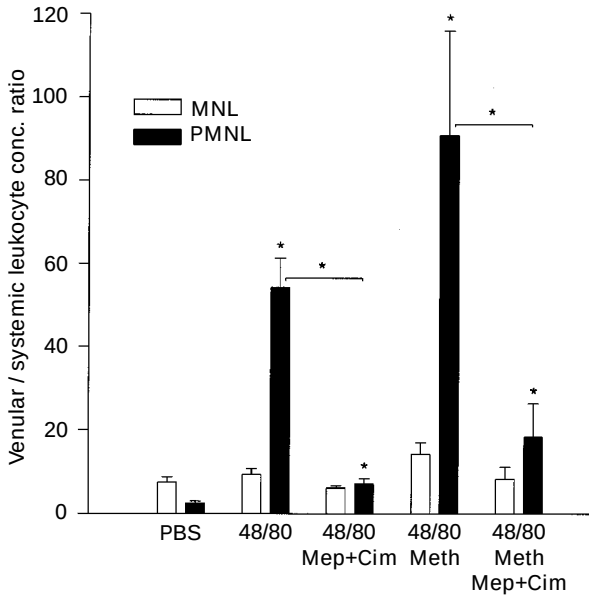

Figure 1 Effects of mepyramine (Mep, 10 mg kg^{-1}) + cimetidine (Cim, 30 mg kg^{-1}) ($n=5$), methysergide (Meth, 0.3 mg kg^{-1}, $n=5$), or a combination of the three drugs ($n=5$) on the increased ratio of mesenteric intravenular to systemic concentration of MNLs and PMNLs induced by i.p. challenge with compound 48/80 (48/80, 50 μg, $n=9$) for 15 min in the rat. Mean values$\pm$s.e.mean are shown. *$P<0.05$ vs PBS or as indicated by bars.

nist, 10 mg kg^{-1}, i.v.), cimetidine (H_2-receptor antagonist, 30 mg kg^{-1}, i.v.), and the 5-HT-receptor antagonist methysergide (0.3 mg kg^{-1}, i.v.). Combined H_1- and H_2-receptor blockade profoundly reduced the PMNL response to compound 48/80 (Figure 1). These results were confirmed in experiments where cimetidine was combined with another H_1-receptor antagonist (diphenhydramine, 10 mg kg^{-1} i.v.), i.e. the venular/systemic PMNL concentration-ratio after cimetidine + diphenhydramine treatment was 9.7 ± 1.3 ($n=5$, $P<0.05$ vs 48/80 alone). In contrast, methysergide did not inhibit the compound 48/80 response (Figure 1), but inhibited the corresponding PMNL response to 10^{-4} M 5-HT (see Figure 2) by $61.8 \pm 7.3\%$ ($n=5$, $P<0.05$ vs 5-HT alone).

Histamine caused venular PMNL accumulation in a strictly dose-dependent manner, with a venular/systemic PMNL concentration-ratio around 50 at histamine 10^{-3} M for 15 min (Figure 2). 5-HT also caused significant venular PMNL accumulation. However, the response was clearly bell shaped (Figure 2), possibly reflecting that 5-HT at higher concentrations (i.e. 10^{-3} M) may have caused vasoconstriction and thereby reduced influx of leukocytes. This was examined by use of intravital microscopy of mesenteric venules before and 5 min after topical agonist challenge. We found that, indeed, 10^{-3} M 5-HT decreased venular blood flow from 15.5 ± 2.88 nl min^{-1} to 5.5 ± 0.97 nl min^{-1} ($n=6$, $P<0.05$). The corresponding values for 5-HT 10^{-4} M were 18.5 ± 2.77 nl min^{-1} and 16.4 ± 2.80 nl min^{-1}, respectively ($n=5$, NS). For comparison, histamine moderately increased venular blood flow at both 10^{-4} M (by $12 \pm 20\%$, $n=5$, $P<0.05$) and 10^{-3} M (by $31 \pm 21\%$, $n=5$, $P<0.05$). However, identical stimulation with compound 48/80 (10 μg ml^{-1}) had no effect on blood flow, i.e. it was $105.8 \pm 21.3\%$ of the prechallenge control value ($n=9$, NS).

In line with the effects of compound 48/80 described above, the MNLs were completely insensitive to the 15 min stimulation with histamine or 5-HT (data not shown). Furthermore, in contrast to histamine and 5-HT, a relatively high dose (10^{-6} M) of the mast cell derived prostanoid PGD_2 had no effect on the venular PMNL concentration (Figure 2).

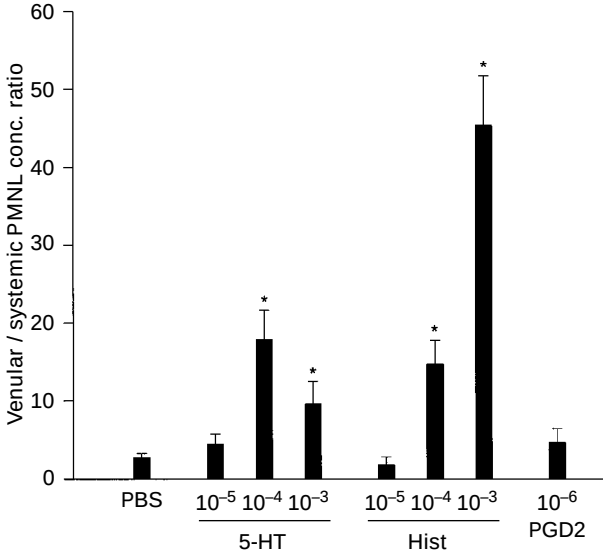

Figure 2 Ratio of mesenteric intravenular to systemic concentration of PMNLs 15 min after i.p. challenge with PBS ($n=9$) or the concentrations of 5-HT indicated ($n=5-13$), histamine (Hist, $n=19$), or PGD_2 ($n=3$) in the rat. Mean values ± s.e.mean are shown. *$P<0.05$ vs PBS.

In control animals, the systemic leukocyte count was $0.73 \pm 0.12 \times 10^6$ ml^{-1} blood ($n=12$), and none of the above i.p. injections or drug treatments significantly changed this parameter (data not shown).

Duration of action of histamine-induced venular PMNL recruitment

The venular/systemic PMNL concentration-ratio in response to histamine (10^{-3} M, i.p.) peaked between 15 min and 1 h, was still significantly elevated at 2 h, and then returned to prechallenge values after 3 h (Figure 3a). As shown in Figure 3b and c, these experiments also illustrate the importance of normalizing evoked changes in venular leukocyte concentration to the systemic leukocyte concentration (at the time of tissue sampling), in order to differentiate between changes in intravascular leukocyte content due to leukocyte-endothelium interactions and those due to variations in the systemic leukocyte count.

We have previously shown that the venular leukocyte accumulation 15 min after i.p. histamine is due to PMNL rolling (Yamaki *et al*., 1998). To examine whether the sustained histamine response was also due to increased rolling, the animals were treated with fucoidin (10 mg kg^{-1}, i.v., 5 min before tissue sampling), which rapidly blocks leukocyte rolling but not firm adhesion *per se* (Lindbom *et al*., 1992). This treatment profoundly reduced both the early and delayed PMNL response to histamine (Figure 3a), without affecting the systemic PMNL count (data not shown). Further in line with the contention that histamine causes a temporary upregulation of PMNL rolling without significant firm adhesion or emigration, there was no significant increase in extravascular PMNLs after i.p. challenge with histamine. Thus, the number of extravascular PMNLs mm^{-2} mesenteric tissue was 41 ± 7 after histamine (10^{-3} M for 3 h) and 21 ± 17 in control animals ($n=4$ in each group, $P=0.372$). For comparison, i.p. challenge with 500 u IL-1β for 3 h resulted in 2883 ± 313 extravascular PMNLs mm^{-2} mesenteric tissue ($n=5$). Furthermore, as with i.p. histamine for 15 min, challenge with histamine for 1–5 h also failed to increase the venular MNL concentration. Thus, the venular/systemic MNL concentration ratios after 15 min of i.p. PBS or 15 min, 1 h, 2 h, 3 h, and 5 h after i.p. histamine (10^{-3} M) were 7.8 ± 1.2, 9.2 ± 0.8, 7.0 ± 2.7, 10.5 ± 2.2, 10.7 ± 1.7, and 9.7 ± 3.2, respectively, with no significant differences between the groups (n same as in Figure 3).

Histamine-receptors involved in histamine-induced venular PMNL recruitment

To examine the role of histamine receptors in the PMNL response to histamine, the animals were pretreated for 10 min with diphenhydramine (H_1-receptor antagonist, 10 mg kg^{-1}, i.v.), cimetidine (H_2-receptor antagonist, 30 mg kg^{-1}, i.v.), or ranitidine (H_2-receptor antagonist, 10 mg kg^{-1}, i.v.). Diphenhydramine inhibited the venular PMNL response to histamine (10^{-3} M, 15 min i.p.) by 52%, while the two H_2-receptor antagonists were inactive in this regard (Figure 4a). However, the combination of cimetidine and diphenhydramine reduced the histamine-induced PMNL rolling by 82% (Figure 4a). Treatment with cimetidine alone was also without effect on the PMNL response to a lower dose of histamine, i.e. the venular/systemic PMNL concentration-ratio in response to histamine 10^{-4} M was 16.2 ± 2.4 ($n=5$) in control animals, and 18.7 ± 4.0 ($n=4$) in the presence of cimetidine.

To explore further the role of histamine-receptors in the PMNL response to histamine, experiments were done with the

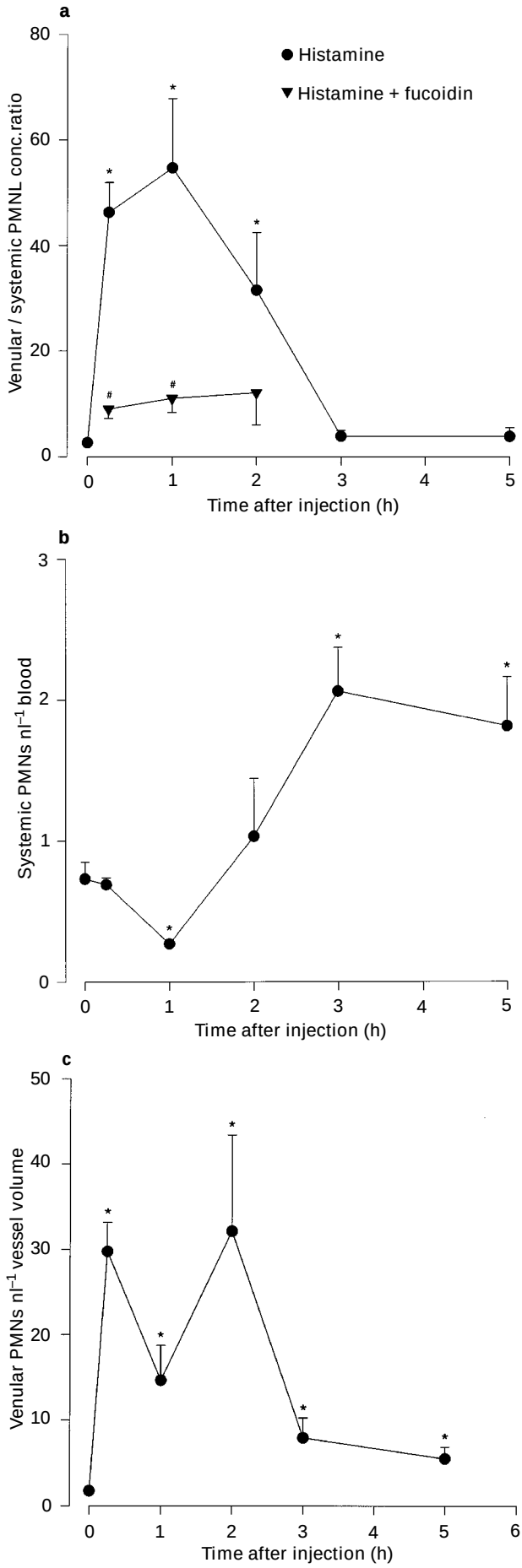

Figure 3 Changes over time in the ratio of mesenteric intravenular to systemic concentrations of PMNLs (a), systemic blood PMNL concentration (b) and venular PMNL concentration (c) at indicated time points after i.p. challenge with histamine (10^{-3} M, $n=4-19$) in the rat. Also shown in (a) is the effect of fucoidin (10 mg kg^{-1}, $n=5-7$) given i.v. 5 min before the animals were killed for tissue sampling. Mean values ± s.e.mean (vertical lines) are shown. $*P<0.05$ vs time 0 (= untreated control animals, $n=12$); $\#P<0.05$ vs corresponding response to histamine alone.

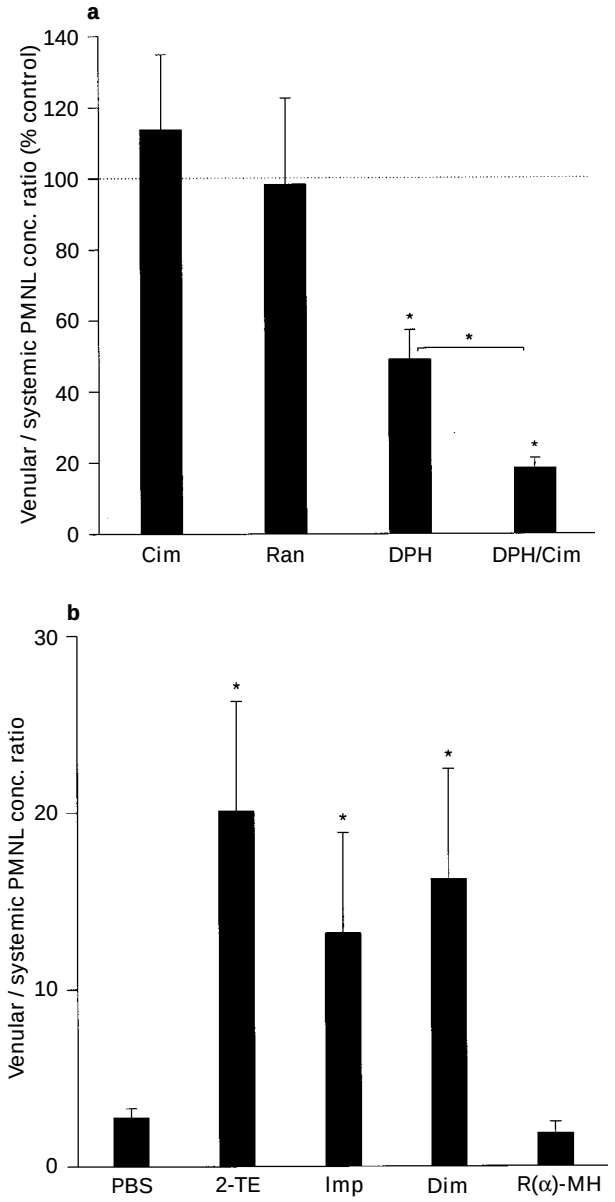

Figure 4 (a) Effects of cimetidine (Cim, 30 mg kg^{-1}, $n=6$), diphenhydramine (DPH, 10 mg kg^{-1}, $n=6$), ranitidine (Ran, 10 mg kg^{-1}, $n=5$), or a combination of cimetidine and diphenhydramine ($n=6$) on histamine-induced increase in the ratio of mesenteric intravenular to systemic PMNL concentration in rat. Mean values ± s.e.mean are shown as % of control response to i.p. histamine (10^{-3} M) alone for 15 min (dashed line). $*P<0.05$ vs control response to histamine alone or as indicated by bar. (b) Ratio of mesenteric intravenular to systemic concentration of PMNLs 15 min after i.p. challenge with PBS ($n=9$), 2-thiazolylethylamine (2-TE; 10^{-3} M, $n=4$), dimaprit (Dim; 10^{-3} M, $n=4$), impromidine (Imp; 10^{-5} M, $n=4$), or R(α)-methylhistamine (R(α)-MH; 10^{-5} M, $n=4$) in the rat. Mean values ± s.e.mean are shown. $*P<0.05$ vs PBS.

H_1-receptor agonist 2-thiazolylethylamine (10^{-3} M, i.p., for 15 min), the two H_2-receptor agonists impromidine (10^{-5} M, i.p., for 15 min) and dimaprit (10^{-3} M, i.p. for 15 min), and the H_3-receptor agonist **R**(α)-methylhistamine (10^{-5} M, i.p., for 15 min). 2-Thiazolylethylamine and the H_2- agonists *per se* increased the venular/systemic PMNL concentration-ratio, whereas **R**(α)-methylhistamine was inactive (Figure 4b). The MNLs were completely insensitive to the different histamine agonists (data not shown).

The concentrations of the histamine agonists were chosen with regard to their potencies relative to histamine at the different histamine-receptors (Hill, 1990), in order to obtain receptor stimulation with the agonists corresponding approximately to the active concentrations of histamine (i.e. histamine $10^{-4}-10^{-3}$ M).

Effects of L-NAME and dexamethasone on histamine-induced venular PMNL recruitment

We also examined the effects of the NO-synthase inhibitor L-NAME (30 mg kg^{-1}, i.v.) on the histamine-induced venular PMNL accumulation. Treatment with L-NAME increased the systemic arterial blood pressure from 78 ± 4.4 to 120 ± 18 mmHg at 10 min, and it remained elevated (133 ± 5.2 mmHg) at 60 min ($n=3$). However, L-NAME treatment did not significantly change the venular PMNL accumulation in response to either 10^{-4} or 10^{-3} M histamine (Table 1). On the other hand, 3 h pretreatment with dexamethasone (1 mg kg^{-1}, i.m.) reduced the PMNL response to histamine by 73% (Table 1).

Effects of dexamethasone on PMNL P-selectin binding and L-selectin expression

To examine possible modes of action of dexamethasone in inhibiting the histamine-induced venular PMNL accumulation (i.e. rolling), the binding of soluble P-selectin to and expression of L-selectin on rat isolated PMNLs was examined by flow cytometry. As shown in Figure 5a, PMNLs from untreated rats bound soluble P-selectin to a much higher degree than that found for soluble L-selectin (used as negative control), with 93% of events for P-selectin labelled cells within the region of positive fluorescence (M2). On the other hand, after dexamethasone treatment (same as above), the binding of P-selectin was almost abolished, with only 10% of events for P-selectin-labelled cells in region M2 (Figure 5b). That the binding of P-selectin was specific was suggested by both the great difference in binding between soluble L- and P-selectin, and by the cation-dependency of P-selectin binding reflected in the pronounced inhibitory action of EDTA (3.4% of events in M2) (Figure 5c). In contrast to P-selectin binding, the expression of L-selectin was unaffected by dexamethasone treatment (Figure 5d). Virtually identical results as those presented in Figure 5 were obtained in two separates experiments with duplicate samples.

Discussion

The method of choice for direct observation of inflammatory leukocyte rolling and adhesion *in vivo* is intravital microscopy of thin exteriorized tissues such as the hamster cheek pouch and the mesentery of different animals. However, as mentioned in the Introduction and by Yamaki *et al.* (1998, accompanying paper), the preparative trauma associated with this technique makes it difficult to examine factors that initiate leukocyte rolling. In the present study, we have examined mechanisms related to histamine-induced leukocyte rolling using a method for leukocyte-endothelium interactions in undisturbed rat mesenteric venules, in which rolling is absent (Yamaki *et al.*, 1998). With this approach, we have shown that i.p. injection of histamine dose-dependently increases the venular PMNL (but not the MNL) concentration up to almost 50 fold the systemic PMNL concentration (Yamaki *et al.*, 1998; this paper). Moreover, this histamine-induced PMNL accumulation was found to be almost entirely dependent on leukocyte rolling.

Histamine is a major mast cell-derived mediator that causes endothelial cell contraction and increased vascular perme-

Table 1 Effects of L-NAME and dexamethasone on histamine-induced PMNL accumulation in rat mesenteric venules

Treatment group	*Venular/systemic PMNL conc. ratio*	n
PBS	2.9 ± 0.5	8
Histamine 0.1 mM	14.6 ± 3.1	12
Histamine 0.1 mM + L-NAME[1]	23.3 ± 4.9	8
Histamine 1 mM	45.4 ± 7.9	23
Histamine 1 mM + L-NAME[1]	57.8 ± 7.8	5
Histamine 1 mM + Dxm[2]	12.2 ± 3.5[3]	4

[1]L-NAME, 30 mg kg^{-1}, i.v., 10 min before i.p. challenge with histamine for 15 min. [2]Dexamethasone (Dxm), 1 mg kg^{-1}, i.m., 3 h before histamine challenge. [3]$P<0.05$ vs histamine 1 mM. Mean values$\pm$s.e.mean are shown.

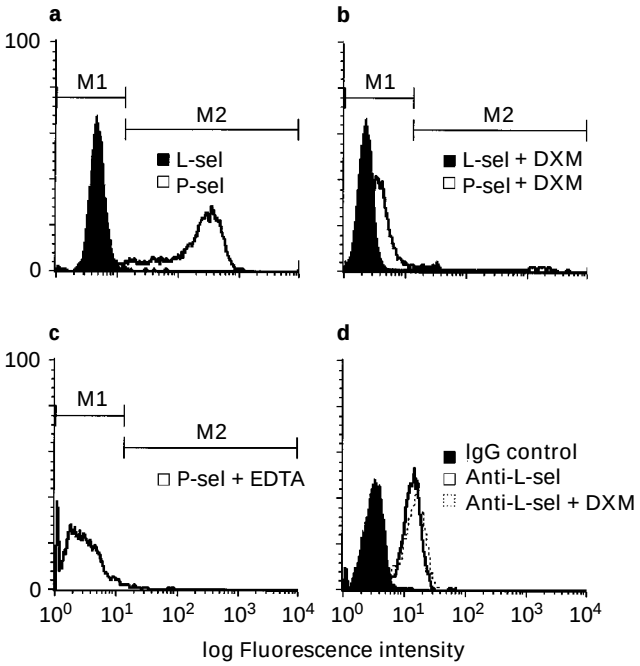

Figure 5 Flow cytometric analysis of effects of dexamethasone treatment on rat isolated PMNL P-selectin binding and L-selectin expression. Binding of soluble P-selectin (P-sel) compared with binding of soluble L-selectin (L-sel) to PMNLs from untreated rats (a), and rats treated with dexamethasone (DXM, 1 mg kg^{-1}, i.m., for 3 h) (b); binding of P-selectin to untreated rat PMNLs in the presence of 10 mM EDTA (c); expression of L-selectin on PMNLs from untreated (Anti-L-sel) and dexamethasone treated rats, with isotype-matched IgG as negative control (IgG control) (d). Region M1 was set to contain $\geqslant$97.5% of fluorescence events for cells labelled with soluble L-selectin (negative control) and region M2 indicates positive fluorescence events (a–c).

ability (Majno & Palade, 1961), up-regulation of P-selectin for initiation of leukocyte rolling (Hattori *et al*., 1989), and potentiation of chemoattractant-induced leukocyte adhesion (Thorlacius *et al*., 1995). Another functionally related mast cell mediator is 5-HT, which also contracts endothelial cells to promote oedema formation (Majno & Palade, 1961). However, it is not known if 5-HT, similar to histamine, can induce leukocyte rolling *in vivo*. Because 5-HT is an important mediator of acute mast cell-dependent oedema in the rat and is approximately 100 times more potent than histamine in this respect (Green *et al*., 1979), we first analysed the relative role of histamine and 5-HT in acute mast cell-dependent venular PMNL recruitment. Mast cell activation was achieved by i.p. injection of compound 48/80, in concentrations known to liberate significant amounts of both histamine and 5-HT from peritoneal and mesenteric mast cells of female Wistar rats (Purcell *et al*., 1989). The striking increase in venular PMNL accumulation after compound 48/80 challenge was prevented by combined H_1- and H_2-receptor antagonist pretreatment. In contrast, the 5-HT antagonist methysergide, which inhibited the PMNL response to exogenous 5-HT, was inactive in this regard. Exogenous 5-HT did express some activity with regard to triggering venular leukocyte accumulation. However, this effect was bell-shaped and the effect of 10^{-3} M 5-HT was five times smaller than that of 10^{-3} M histamine. One likely explanation for this difference between exogenous histamine and 5-HT at 10^{-3} M was that, in contrast to histamine, 5-HT caused vasoconstriction and thus reduced the influx of leukocytes. The apparent lack of involvement of endogenous 5-HT in the mast cell-dependent leukocyte response could not be explained by such a blood flow-dependent mechanism, because the challenge with compound 48/80 caused no change in venular blood flow. Another mediator that is released in significant amounts from activated mast cells is PGD_2 (Lewis *et al*., 1982). However, in our rat model, a relatively high concentration of this prostanoid did not cause overt venular PMNL accumulation. Our findings in the rat thus suggest that while 5-HT is crucial for mast cell-dependent plasma extravasation in the rat (Green *et al*., 1979; Raud *et al*., 1995), initiation of PMNL rolling is mainly dependent on the release of histamine.

By use of intravital microscopy, we have previously shown that, in addition to increased rolling, challenge with compound 48/80 also causes significant firm leukocyte adhesion (Thorlacius *et al*., 1994), most likely due to release of mast cell-derived chemotactic factors, such as platelet-activating factor and different leukocyte-attracting cytokines (Harvima & Schwartz 1993). Thus, knowing that acute challenge with histamine *per se* causes rolling but little or no firm adhesion in rat mesenteric venules (Thorlacius *et al*., 1994; 1995; Yamaki *et al*., 1998; this paper), it may seem surprising that the PMNL accumulation after mast cell activation was almost entirely inhibited by antihistamine treatment. However, because leukocyte rolling *in vivo* has been shown to be a prerequisite for firm leukocyte adhesion (Lindbom *et al*., 1992), a likely explanation for the very powerful effect of antihistamine treatment on leukocyte accumulation is that free-flowing leukocytes in the microcirculation do not respond to mast cell chemoattractants unless first stimulated to roll by histamine. This is supported by our previous observation that inhibition of compound 48/80-induced leukocyte rolling in the rat mesentery by an anti-P-selectin antibody proportionally reduces rolling and firm adhesion (Thorlacius *et al*., 1994). Moreover, also using intravital microscopy, Gaboury *et al*. (1995) have found a similar proportional relationship between mast cell-dependent rolling and adhesion in rats treated with an H_1-receptor antagonist. In addition, we have demonstrated a striking synergism between histamine and chemotactic factors in the rat mesentery (Thorlacius *et al*., 1995).

A discussed above, histamine release is obviously of great importance for initial leukocyte recruitment in acute mast cell-dependent inflammation, and our next step was to characterize the relative importance of different histamine-receptors in this process. This particular issue has recently been addressed in three intravital microscopic studies in rat mesentery, where the authors reached partly contradictory conclusions with regard to the involvement of H_1- and H_2-receptors in histamine-induced rolling. Thus, two studies showed that the leukocyte rolling in response to histamine was abolished by the H_1-antagonist diphenhydramine and unaffected by the H_2-antagonist cimetidine (Asako *et al*., 1994; Kubes & Kanwar, 1994), while the third study, with diphenhydramine and the H_2-antagonist ranitidine, demonstrated opposite effects (Ley, 1994). It is difficult to explain these discrepancies. However, it is possible that certain differences in experimental design and variations in 'spontaneous' preparation-induced rolling may have affected the results (see also Yamaki *et al*., 1998). In our present experiments, with no interference of 'spontaneous' rolling, we found that histamine-induced leukocyte accumulation involved both H_1- and H_2-receptors. Thus, diphenhydramine reduced the histamine response by 50%, while cimetidine and ranitidine *per se* were inactive. Interestingly, a combination of diphenhydramine and cimetidine further reduced the histamine-induced PMNL accumulation by 80%. The notion that H_1- and H_2-receptors together mediated the PMNL response to histamine was strengthened by experiments showing that challenge with either an H_1-receptor agonist or two different H_2-receptor agonists *per se* was sufficient to trigger venular leukocyte accumulation. In contrast, an H_3-receptor agonist was inactive in this regard. Our finding that histamine-induced leukocyte accumulation (i.e. rolling) involves both H_1- and H_2-receptors correlates with a number of previous studies of oedema and vasodilator responses to histamine in man as well as in different animals (e.g. Marks & Greaves, 1977; Cheng *et al*., 1979; Smith *et al*., 1980; Koo, 1983). Such a dual H_1- and H_2-receptor involvement may also help explain the contradictory intravital microscopic findings on histamine receptors and leukocyte rolling mentioned above.

The approach we used to study leukocyte behaviour in undisturbed venules (exhibiting no 'spontaneous' rolling) is very useful for analysing the kinetics of agonist-induced leukocyte-endothelium interactions. With regard to such kinetics, the histamine-induced leukocyte accumulation was found to peak between 15 min and 1 h, was still significantly elevated at 2 h, and then returned to and remained at prechallenge values from 3 h and onwards. Moreover, in contrast to i.p. injection of interleukin-1β (IL-1β), the challenge with histamine was not associated with leukocyte extravasation. By use of previously described short-term treatment with fucoidin (Yamaki *et al*., 1998), which blocks rolling but not firm leukocyte adhesion *per se* (Lindbom *et al*., 1992), we could also show that the histamine-induced venular leukocyte recruitment was due to rolling rather than firm adhesion over the entire 0–3 h period. This is in contrast to observations by Kubes & Kanwar (1994) and Suzuki *et al*. (1995), who obtained a marked and somewhat delayed increase in firm leukocyte adhesion by topical histamine in the rat mesentery exteriorised for intravital microscopy, possibly as a result of synergism between the exogenous histamine and endogenous chemoattractants released by the surgical procedure.

Based on a number of studies in different experimental systems, it is reasonable to believe that leukocyte rolling during the 'late phase' of acute inflammation may involve E-selectin and VCAM-1 (Montefort *et al*., 1993; Carlos & Harlan, 1994; Berlin *et al*., 1995; Kunkel *et al*., 1996). Moreover, it is well established that the expression of these inducible endothelial adhesion molecules can be triggered by tumour necrosis factor-α (TNF-α) and IL-4 (Montefort *et al*., 1993; Carlos & Harlan, 1994), both of which are released from mast cells (Harvima & Schwartz, 1993). Therefore, knowing that the synthesis and expression of E-selectin and VCAM-1 occurs with a time lag of a few hours (Montefort *et al*., 1993; Carlos & Harlan, 1994), it seems likely that the presently observed 2–3 h duration of histamine-induced rolling serves to promote endothelial tethering of leukocytes during the acute phase of allergic inflammation until adhesion molecules that mediate 'late rolling' have been upregulated. Furthermore, assuming that the presently observed histamine response is P-selectin-dependent (discussed in Yamaki *et al*., 1998), it appears that histamine-induced P-selectin expression *in vivo* is much more long-lasting than that observed in endothelial cell cultures, where increased P-selectin on the cell surface returns to baseline levels within 30 min after histamine challenge (Hattori *et al*., 1989; Foreman *et al*., 1994). However, at present, we cannot exclude the possibility that the delayed PMNL-response to histamine was mediated by receptors other than P-selectin (e.g. L-selectin).

When given i.p. for 15 min, histamine did not increase the venular accumulation of MNLs, and, as shown previously (Yamaki *et al*., 1998), the same is true for the first 15 min after preparation of the rat mesentery for intravital microscopy (Yamaki *et al*., 1998). However, while histamine challenge for up to 5 h failed to recruit MNLs, the surgical preparation increases the venular accumulation of MNLs after 30 min (Yamaki *et al*., 1998). These findings thus illustrate that the effect of histamine on inflammatory leukocyte recruitment is highly selective for PMNLs, and, consequently, that mediators other than histamine are responsible for triggering the initial MNL interactions with endothelium during intravital microscopy.

The concentrations of exogenous histamine required to evoke PMNL recruitment in the rat mesentery ($\geqslant 10^{-4}$ M) may appear high or even 'unphysiological' (Yamaki *et al*., 1998; this paper). However, because the mast cell-dependent leukocyte accumulation was abolished by antihistamines and mimicked by histamine at 10^{-3} M (but not 10^{-4} M), it seems that mast cell activation may indeed lead to local tissue concentrations of histamine of 10^{-3} M or more. In fact, such high levels of tissue histamine have been suggested to occur in models for antigen-induced inflammation (Plaut, 1979) and are also in line with *in vitro* experiments on endothelial histamine-induced P-selectin expression and neutrophil adherence (Foreman *et al*., 1994). With regard to the lower doses (10^{-6}–10^{-5} M) of histamine required to provoke leukocyte rolling in the rat mesentery obtained by Ley (1994) and Asako *et al*. (1994), it may be speculated that the smaller amounts of exogenous histamine may have acted in synergy with endogenous mediators liberated due to the surgical preparation required for the microscopic observation. This is supported by intravital microscopic findings by Kubes & Kanwar (1994) who found 10^{-4} M to be the lowest dose of histamine that significantly increased rolling in preparations where 'spontaneous' rolling had been pharmacologically reduced.

In addition to antihistamines, another common therapy in acute allergic inflammation is the use of glucocorticoids. These potent anti-inflammatory agents are known to inhibit effectively leukocyte recruitment (Schleimer, 1993), at least partly by interference with the transcription of adhesion molecules such as E-selectin and ICAM-1 (Cronstein *et al*., 1992). Furthermore, glucocorticoids are known to inhibit the direct effect of histamine on increased vascular permeability (Tsurufuji & Ohuchi, 1989). In the present study, we showed that the potent steroid dexamethasone also markedly attenuates the rapid histamine-induced PMNL accumulation (i.e. rolling) in small venules. This suggests that inhibition of histamine-induced rolling may be an additional mode of action for the therapeutic effects of steroids in acute inflammatory disorders. Our findings extend the intravital microscopic observations by Suzuki *et al*. (1995); they found that hydrocortisone inhibits the somewhat delayed firm leukocyte adhesion that occurs when the exteriorised rat mesentery is superfused with histamine (see above). In the latter study, it was also argued that glucocorticoids may act via inhibition of endothelial P-selectin expression. In the present study, we found an additional mode of steroid action potentially related to histamine and leukocyte function. Thus, we observed that dexamethasone almost abolished binding of soluble P-selectin to rat PMNLs. This is in agreement with previous *in vitro* observations that histamine-induced neutrophil adherence to endothelium, a P-selectin-dependent event, is inhibited by dexamethasone treatment of the neutrophils, but not by only treating the endothelium (Watanabe *et al*., 1991). The detailed molecular mechanism involved in the dexamethasone-induced inhibition of PMNL binding to P-selectin was not addressed in the present study. However, it may be speculated that glucocorticoids alter the expression or function of a rat equivalent to P-selectin glycoprotein ligand-1 (PSGL-1), a high affinity P-selectin ligand demonstrated on human leukocytes (e.g. Saku *et al*., 1993; Moore *et al*., 1995).

It is well established that histamine can trigger NO production in endothelial cells (Moncada, 1992). Moreover, recent evidence suggests that histamine may increase vascular permeability through the production of NO, which activates guanylate cyclase (Yuan *et al*., 1993). Accordingly, several *in vivo* studies have shown that specific inhibitors of the NO-synthase can inhibit histamine-induced oedema formation in different species (Teixeira *et al*., 1993; Mayhan 1994; Paul *et al*., 1994). It was therefore of interest to examine if the presently observed venular PMNL response to histamine also involved an NO pathway. However, despite using a high dose of the NO-synthase inhibitor L-NAME, we observed no reduction in the histamine-induced mesenteric leukocyte accumulation. Thus, if the NO-guanylate cyclase pathway is indeed crucial for histamine-induced oedema formation, our findings suggest that the intracellular processes that trigger endothelium-dependent rolling and increased permeability are somehow dissociated. In this context, it is also worth noting that endogenous NO has been suggested to reduce leukocyte rolling and adhesion (Kubes *et al*., 1991; Davenpeck *et al*., 1994), i.e. inhibition of NO-synthase was found to cause a slowly developing increase in 'spontaneous' leukocyte rolling and adhesion, as studied by intravital microscopy (time lag $\approx$1 h). However, such a modulating effect of endogenous NO did not appear to play a significant role in the rapid histamine-induced leukocyte accumulation observed in the present study.

In summary, using an *in vivo* model for leukocyte recruitment in undisturbed venules of the rat mesentery, we have shown that initial leukocyte recruitment after mast cell activation is critically dependent on histamine release. The cellular response to histamine, which was due to leukocyte rolling, was dose-dependent, involved activation of both H_1- and H_2-receptors, was specific for PMNLs, and lasted for 2–3 h. Moreover, this histamine-induced PMNL recruitment did not appear to involve an NO-dependent pathway, but was sensitive to

glucocorticoid treatment which apparently inhibited the expression or function of leukocytic P-selectin ligand(s).

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References

ASAKO, H., KUROSE, I., WOLF, R., DEFREES, S., ZHENG, Z.L., PHILLIPS, M.L., PAULSON, J.C. & GRANGER, D.N. (1994). Role of H_1 receptors and P-selectin in histamine-induced leukocyte rolling and adhesion in postcapillary venules. *J. Clin. Invest.*, **93,** 1508 – 1515.

BERLIN, C., BARGATZE, R.F., CAMPBELL, J.J., VON ANDRIAN, U.H., SZABO, M.C., HASSLEN, S.R., NELSON, R.D., BERG, E.L., ERLANDSEN, S.L. & BUTCHER, E.C. (1995). Alpha 4 integrins mediate lymphocyte attachment and rolling under physiologic flow. *Cell,* **80,** 413 – 422.

CARLOS, T.M. & HARLAN, J.M. (1994). Leukocyte-endothelial adhesion molecules. *Blood,* **84,** 2068 – 2101.

CHENG, H.C., REAVIS, Jr, O.K., MUNRO, N.L. & WOODWARD, J.K. (1979). Cutaneous vascular histamine H_1 and H_2 receptors in the guinea-pig: the histamine skin wheal as a cutaneous vascular model. *Arch. Int. Pharmacodyn. Ther.,* **240,** 214 – 219.

CRONSTEIN, B.N., KIMMEL, S.C., LEVIN, R.I., MARTINIUK, F. & WEISSMANN, G. (1992). A mechanism for the antiinflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1. *Proc. Natl. Acad. Sci. U.S.A.,* **89,** 9991 – 9995.

DAVENPECK, K.L., GAUTHIER, T.W. & LEFER, A.M. (1994). Inhibition of endothelial-derived nitric oxide promotes P-selectin expression and actions in the rat microcirculation. *Gastroenterology,* **107,** 1050 – 1058.

FOREMAN, K.E., VAPORCIYAN, A.A., BONISH, B.K., JONES, M.L., JOHNSON, K.J., GLOVSKY, M.M., EDDY, S.M. & WARD, P.A. (1994). C5a-induced expression of P-selectin in endothelial cells. *J. Clin. Invest.,* **94,** 1147 – 1155.

FOXALL, C., WATSON, S.R., DOWBENKO, D., FENNIE, C., LASKY, L.A., KISO, M., HASEGAWA, W., ASA, D. & BRANDLEY, B.K. (1992). The three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl $Lewis^x$ oligosaccharide. *J. Cell Biol.,* **117,** 895 – 902.

GABOURY, J.P., JOHNSTON, B., NIU, X.F. & KUBES, P. (1995). Mechanisms underlying acute mast cell-induced leukocyte rolling and adhesion *in vivo*. *J. Immunol.,* **154,** 804 – 813.

GREEN, A.F., GARLAND, L.G. & HODSON, H.F. (1979). Antagonists of histamine, 5-hydroxytryptamine and SRS-A. In *Anti-Inflammatory Drugs*. ed. Vane, J.R. & Ferreira, S.H. pp. 415 – 466. Berlin: Springer-Verlag.

HARVIMA, R.J. & SCHWARTZ, L.B. Mast cell-derived mediators. (1993). In *The Handbook of Immunopharmacology: Immunopharmacology of Mast Cells and Basophils*. ed. Foreman, J.C. pp. 115 – 138. London: Academic Press.

HATTORI, R., HAMILTON, K.K., FUGATE, R.D., MCEVER, R.P. & SIMS, P.J. (1989). Stimulated secretion of endothelial von Willebrand factor is accompanied by rapid redistribution to the cell surface of the intracellular granule membrane protein GMP-140. *J. Biol. Chem.,* **264,** 7768 – 7771.

HILL, S.J. (1990). Distribution, properties, and functional characteristics of three classes of histamine receptor. *Pharmacol. Rev.,* **42,** 45 – 83.

KOO, A. (1983). *In vivo* characterization of histamine H_1- and H_2-receptors in the rat stomach microcirculation. *Br. J. Pharmacol.,* **78,** 181 – 189.

KUBES, P. & KANWAR, S. (1994). Histamine induces leukocyte rolling in post-capillary venules. A P-selectin-mediated event. *J. Immunol.,* **152,** 3570 – 3577.

KUBES, P., SUZUKI, M. & GRANGER, D.N. (1991). Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc. Natl. Acad. Sci. U.S.A.,* **88,** 4651 – 4655.

KUNKEL, E.J., JUNG, U., BULLARD, D.C., NORMAN, K.E., WOLITZKY, B.A., VESTWEBER, D., BEAUDET, A.L. & LEY, K. (1996). Absence of trauma-induced leukocyte rolling in mice deficient in both P-selectin and intercellular adhesion molecule 1. *J. Exp. Med.,* **183,** 57 – 65.

LAWRENCE, M.B. & SPRINGER, T.A. (1991). Leukocytes roll on a selectin at physiologic flow rates: distinction from the prerequisite for adhesion through integrins. *Cell,* **65,** 859 – 873.

LEWIS, R.A., SOTER, N.A., DIAMOND, P.T., AUSTEN, K.F., OATES, J.A. & ROBERTS, L.J., 2nd. (1982). Prostaglandin D_2 generation after activation of rat and human mast cells with anti-IgE. *J. Immunol.,* **129,** 1627 – 1631.

LEY, K. (1994). Histamine can induce leukocyte rolling in rat mesenteric venules. *Am. J. Physiol.,* **267,** H1017 – H1023.

LINDBOM, L., XIE, X., RAUD, J. & HEDQVIST, P. (1992). Chemoattractant-induced firm adhesion of leukocytes to vascular endothelium *in vivo* is critically dependent on initial leukocyte rolling. *Acta Physiol. Scand.,* **146,** 415 – 421.

MAJNO, G. & PALADE, G.E. (1961). Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability: an electron microscopic study. *J. Biophys. Biochem. Cytol.,* **11,** 571 – 605.

MAYHAN, W.G. (1994). Nitric oxide accounts for histamine-induced increases in macromolecular extravasation. *Am. J. Physiol.,* **266,** H2369 – H2373.

MARKS, R. & GREAVES, M.W. (1977). Vascular reactions to histamine and compound 48/80 in human skin: suppression by a histamine H_2-receptor blocking agent. *Br. J. Clin. Pharmacol.,* **4,** 367 – 369.

MONCADA, S. (1992). The 1991 Ulf von Euler Lecture. The L-arginine: nitric oxide pathway. *Acta Physiol. Scand.,* **145,** 201 – 227.

MONTEFORT, S., HOLGATE, S.T. & HOWARTH, P.H. (1993). Leucocyte-endothelial adhesion molecules and their role in bronchial asthma and allergic rhinitis. *Eur. Respir. J.,* **6,** 1044 – 1054.

MOORE, K.L., PATEL, K.D., BRUEHL, R.E., FUGANG, L.I., JOHNSON, D.A., LICHENSTEIN, H.S., CUMMINGS, R.D., BAINTON, D.F. & MCEVER, R.P. (1995). P-selectin glycoprotein ligand-1 mediates rolling of human neutrophils on P-selectin. *J. Cell Biol.,* **128,** 661 – 671.

PAUL, W., DOUGLAS, G.J., LAWRENCE, L., KHAWAJA, A.M., PEREZ, A.C., SCHACHTER, M. & PAGE, C.P. (1994). Cutaneous permeability responses to bradykinin and histamine in the guinea-pig: possible differences in their mechanism of action. *Br. J. Pharmacol.,* **111,** 159 – 164.

PLAUT, M. (1979). Histamine, H_1 and H_2 antihistamines, and immediate hypersensitivity reactions. *J. Allergy Clin. Immunol.,* **63,** 371 – 375.

PURCELL, W.M., COHEN, D.L. & HANAHOE, T.H. (1989). Comparison of histamine and 5-hydroxytryptamine content and secretion in rat mast cells isolated from different anatomical locations. *Int. Arch. Allergy Appl. Immunol.,* **90,** 382 – 386.

RAUD, J., KONRAD, D. & DAHLÉN, S.E. (1995). Delayed anti-inflammatory action of nedocromil sodium in the rat paw is dependent on *de novo* protein synthesis. *Eur. J. Pharmacol.,* **282,** 207 – 211.

SAKU, D., CHANG, X.J., BARONE, K.M., VACHINO, G., WHITE, H.M., SHAW, G., VELDMAN, G.M., BEAN, K.M., AHERN, T.J., FURIE, B., CUMMING, D.A. & LARSEN, G.R. (1993). Expression cloning of a functional glycoprotein ligand for P-selectin. *Cell,* **75,** 1179 – 1186.

SCHLEIMER, R.P. (1993). An overview of glucocorticoid anti-inflammatory actions. *Eur. J. Clin. Pharmacol.,* **45** (suppl. 1), S3 – S7.

SMITH, J.A., MANSFIELD, L.E. & DE SHAZO, R.D. (1980). An evaluation of the pharmacologic inhibition of the immediate and late cutaneous reaction to allergen. *J. Allergy Clin. Immunol.,* **65,** 118 – 121.

SUZUKI, H., ZWEIFACH, B.W., FORREST, M.J. & SCHMID-SCHÖNBEIN, G.W. (1995). Modification of leukocyte adhesion in spontaneously hypertensive rats by adrenal corticosteroids. *J. Leukocyte Biol.,* **57,** 20 – 26.

TEIXEIRA, M.M., WILLIAMS, T.J. & HELLEWELL, P.G. (1993). Role of prostaglandins and nitric oxide in acute inflammatory reactions in guinea-pig skin. *Br. J. Pharmacol.,* **110,** 1515 – 1521.

THORLACIUS, H., RAUD, J., ROSENGREN-BEEZLEY, S., FORREST, M.J., HEDQVIST, P. & LINDBOM, L. (1994). Mast cell activation induces P-selectin-dependent leukocyte rolling and adhesion in postcapillary venules *in vivo*. *Biochem. Biophys. Res. Commun.,* **203,** 1043 – 1049.

THORLACIUS, H., RAUD, J., XIE, X., HEDQVIST, P. & LINDBOM, L. (1995). Microvascular mechanisms of histamine-induced potentiation of leukocyte adhesion evoked by chemoattractants. *Br. J. Pharmacol.,* **116,** 3175 – 3180.

TOGIAS, A., NACLERIO, R.M., PROUD, D., PIPKORN, U., BASCOM, R., ILIOPOULOS, O., KAGEY-SOBOTKA, A., NORMAN, P.S. & LICHTENSTEIN, L.M. (1988). Studies on the allergic and nonallergic nasal inflammation. *J. Allergy Clin. Immunol.,* **81,** 782 – 790.

TSURUFUJI, S. & OHUCHI, K. (1989). *In vivo* models of inflammation: a review with special reference to the mechanism of action of glucocorticoids. In *Anti-Inflammatory Steroid Action*. ed. Schleimer, R.P., Claman, H.N. & Oronsky, A.L. pp. 259 – 279. San Diego: Academic Press.

WATANABE, M. YAGI, M., OMATA, M., HIRASAWA, N. MUE, S., TSURUFUJI, S. & OHUCHI, K. (1991). Stimulation of neutrophil adherence to vascular endothelial cells by histamine and thrombin and its inhibition by PAF antagonists and dexamethasone. *Br. J. Pharmacol.,* **102,** 239 – 245.

WATSON, S.R., IMAI, Y., FENNIE, C., GEOFFREY, J.S., ROSEN, S.D. & LASKY, L.A. (1990). A homing receptor IgG chimera as a probe for adhesive ligands of lymph node high endothelial venules. *J. Cell Biol.,* **110,** 2221 – 2229.

YAMAKI, K., LINDBOM, L., THORLACIUS, H., HEDQVIST, P. & RAUD, J. (1998). An approach for studies of mediator-induced leukocyte rolling in the undisturbed microcirculation of the rat mesentery. *Br. J. Pharmacol.,* **123,** 381 – 389.

YUAN, Y., GRANGER, H.J., ZAWIEJA, D.C., DEFILY, D.V. & CHILIAN, W.M. (1993). Histamine increases venular permeability via a phospholipase C-NO synthase-guanylate cyclase cascade. *Am. J. Physiol.,* **264,** H1734 – H1739.

ZWEIMAN, B. (1988). Mediators of allergic inflammation in the skin. *Clin. Allergy,* **18,** 419 – 433.

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