Int. J. Exp. Path. (1994) **75**, 43–50

Mast-cell histamine is angiogenic through receptors for histamine$_1$ and histamine$_2$

JAN SÖRBO, ANDERS JAKOBSSON AND KLAS NORRBY
Department of Pathology, Göteborg University, Sahlgren Hospital, Göteborg, Sweden

Received for publication 14 July 1993
Accepted for publication 29 September 1993

Summary. The activation of mast-cells *in situ* induces angiogenesis in normally vascularized, adult mammalian tissue. Since the secreting mast-cell characteristically releases histamine, we studied the possible role of histamine in the outcome of mast-cell mediated angiogenesis using the rat mesenteric window assay. One H_1-receptor antagonist, brompheniramine maleate (BPA), and one H_2-receptor antagonist, metiamide, were separately administered systemically (s.c.) at non-toxic doses during the period of angiogenesis induction. Angiogenesis was effected by i.p. injections of the mast-cell secretagogue compound 48/80 for 5 consecutive days. The animals were killed 14 days after the start of the i.p. and s.c. treatment, close to the middle of the expanding angiogenic phase of the angiogenic reaction studied. Angiogenesis was quantified in terms of (a) the *number of vessel profiles per unit tissue length* (No/UL), which reflects mainly the degree of branching and/or tortuosity, (b) the *relative vascularized area* (VA), which is a measure of spatial extension, and (c) the *vascular density* (VD), a measure of vessel density per unit area of vascularized tissue. Whereas BPA significantly suppressed No/UL, metiamide significantly reduced No/UL and VD in statistical terms suggesting that endogenous mast-cell histamine is angiogenic through both H_1- and H_2-receptors. This appears to be the first paper to report that the occupancy of H_2-receptors is angiogenic.

Keywords: angiogenesis, histamine, H_1-receptor antagonist, H_2-receptor antagonist, mast cells, quantification, rat, mesentery

Angiogenesis, the formation of new blood vessels, is a complex tissue reaction in which many types of cell take part. Physiological angiogenesis is stringently controlled and apparently occurs only in adulthood in females, in the formation of the corpus luteum and the growth of the endometrium (Hobson & Denekamp 1984; Folkman 1992). In reparative and pathological conditions, however, angiogenesis can be induced promptly, as occurs in tissue repair, inflammatory conditions and tumour growth (Folkman 1990).

Correspondence: Professor Klas Norrby, Department of Pathology, Sahlgren Hospital, S-413 45 Göteborg, Sweden.

The connective tissue mast-cell, a highly specialized secretory cell and the main repository for histamine in the body, is often topographically associated with microvessels (Cowen *et al.* 1979; Rakusan *et al.* 1990). The mast-cell is capable of responding to a wide range of endogenous and exogenous secretagogues or stimuli by releasing pre-formed, rapidly eluted mediators such as histamine, serotonin (in some species including the rat); pre-formed granule associated factors such as heparin, chymase, plasminogen activator; and newly generated mediators such as prostaglandins, leukotrienes and a large number of cytokines (Gordon *et al.* 1990; Norrby &

Woolley 1993). Mast-cells play important roles in inflammatory reactions, wound repair and the growth of several human and experimental tumours (Enerbäck & Norrby 1989; Meininger & Zetter 1992; Norrby & Woolley 1993). As reported previously, the selective activation of mast-cells *in situ* induces vigorous angiogenesis in adult mammalian tissue (Norrby *et al.* 1986; 1989; 1990a). This finding was subsequently confirmed in the chick chorioallantoic membrane (CAM), where mast-cell secretion increases the vascularity and tortuosity of the organogenically developing vessels (Clinton *et al.* 1988; Duncan *et al.* 1992).

Recently, we demonstrated that systemic treatment with clemastine, a histamine$_1$-receptor antagonist, suppresses mast-cell mediated angiogenesis by significantly hampering the microvascular spatial expansion, as assessed by the rat mesenteric window assay (Sörbo & Norrby 1992). The aim of the present study was further to elucidate the role of endogenous histamine in mast-cell mediated angiogenesis using the same assay. In the present paper, the systemic effects of another H_1-receptor antagonist, brompheniramine maleate, and an H_2-receptor antagonist, metiamide, were studied. Angiogenesis was assayed using technically independent procedures in terms of the number of vessels per unit length of the microtome-sectioned mesenteric window and the relative vascularized area, as well as the vascular density in spreads of intact mesenteric window.

Material and methods

Animals

Adult albino male Sprague–Dawley rats (Alab AB, Sollentuna, Sweden) weighing between 190 and 228 g at the start of the experiments were used. They had an unlimited supply of standard pellets and water and two animals were kept in each cage under standardized conditions (Norrby *et al.* 1986). The body weight of the animals was monitored throughout the experiments.

Test tissue

The true rat mesentery, extending from the dorsal peritoneal wall to the small gut, contains some 40–45 somewhat triangularly-shaped 'window'-like connective tissue membranes framed by vascularized fatty arcades. These mesenteric windows are extremely thin and natively measure only 5–10 μm (Norrby & Enestrom 1984). The test tissue is covered on both sides by a single layer of mesothelial cells residing on a basement membrane and contains fibroblasts, mast-cells, macrophages and occasional leucocytes, and the extracellular matrix is rich in collagen fibres. The fact that the test tissue is adult and vascularized (Norrby *et al.* 1986) suggests that the assay is pertinent to those tissues that display clinically relevant, angiogenesis related lesions. The histamine content in rat connective tissue mast-cells is about 9% of the cellular dry weight (w/w) (Mellblom & Enerbäck 1979) and in the test tissue, the mast-cell is the main, if not the only, source of histamine.

Agents

Compound 48/80 (Sigma) was used as a highly selective mast-cell secretagogue. The drug was injected i.p.; 48/80 *per se* appears to be mitogenically (Franzén & Norrby 1980) and angiogenically (Norrby *et al.* 1986) inert in the mesenteric window of guinea-pigs, the mast-cells of which are not responsive to the drug.

Brompheniramine maleate (BPA; generously supplied by Draco AB, Lund, Sweden), an H_1-receptor antagonist, was dissolved in saline (0.9% NaCl; v/w) and was given at doses of 0.150, 0.225 and 3.000 mg/kg b.w. per s.c. injection (0.1 ml/100 g b.w.). An injected single dose of 0.07–0.30 mg/kg or an oral dose of 0.06 mg/kg is recommended to induce an H_1-receptor blocking effect for 4–6 hours in adult humans (Garrison 1990).

Metiamide (generously supplied by Smith, Kline & French Laboratories Ltd, Welwyn Garden City, England), a potent H_2-receptor antagonist, dissolved in saline, was used at doses of 2.0, 3.0 and 75 mg/kg b.w. per s.c. injection (0.2 ml/100 g b.w.). An approximate oral ED-50 of 6.25 mg/kg has been estimated for the inhibition of basal gastric secretion in rats and, in toxicity tests, daily oral infusions of 375 mg/kg of metiamide produce no tissue changes or local reactions (Black *et al.* 1973).

Both histamine-receptor antagonists were applied s.c. to imitate a clinical situation of parenteral drug administration. In so doing, any possible interaction with the mast-cell secretagogue compound 48/80 was eliminated. The s.c. injections were given at 0700 and 1400 h, i.e. 1 hour before the animals received an i.p. injection of compound 48/80. Controls received s.c. injections of the saline vehicle.

Induction of mast-cell mediated angiogenesis

Compound 48/80 was injected i.p. twice daily for 4.5 days, as described elsewhere (Norrby *et al.* 1986). This treatment induces a strong angiogenic reaction (Norrby *et al.* 1989; 1990a), and releases some 60% of the histamine in the test tissue (Norrby *et al.* 1989). The animals were killed at day 14 after the start of the angiogenic treatment, which is approximately in the middle of the angiogenic phase (Norrby *et al.* 1986).

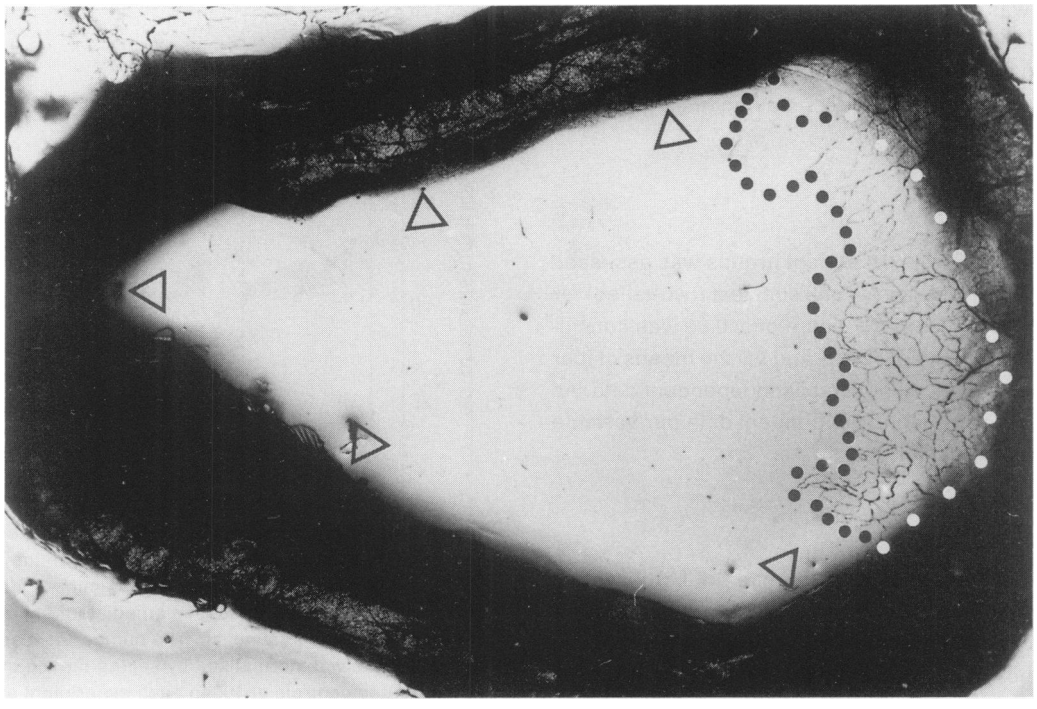

Figure 1. Photomacrograph of a spread of an intact mesenteric window. A Wild M 420 photomacroscope, equipped with an apochromatic Apozoom objective was used. Triangles indicate the border between the mesenteric window and the framing fatty arcades. Since this border is distinct, it is easy to measure the entire window area. The dotted line surrounds the vascularized area. The blood vessels were visualized by a terminal infusion of an i.v. ink–gelatine solution. The total area of this window was 1.30 cm^2 and the vascularized area was 14.8%.

Quantification of the angiogenic response

Three experiments were performed. In the initial experiment, a central part of the mesenteric window was excised, fixed, methacrylate-embedded, cut into 3-μm-thick sections perpendicular to the membrane surface and stained with toluidine blue (Norrby *et al.* 1986). In the second experiment, the tissue was sampled at a peripheral part of the mesenteric window, at either of the two corners close to the small gut. The number of vessel profiles per unit length of microtome-sectioned mesenteric window (No/UL) was counted microscopically at $\times$ 400–600, which is necessary reproducibly to discern the small, newly formed vessels (Norrby *et al.* 1990b). No/UL reflects to a great extent the degree of branching and the degree of tortuosity of the microvasculature. Four windows from each animal were analysed.

In the third experiment we used whole, intact mesenteric window spreads prepared on objective slides (Enerbäck *et al.* 1976). A terminal i.v. ink–gelatine solution was infused to visualize the vasculature in the windows (Norrby *et al.* 1990a). Four windows from each animal were excised and the specimens were analysed morphometrically in terms of the relative vascularized area (VA) from a projected microscopic image (Norrby *et al.* 1990a). A macrophotograph of a window spread is shown in Figure 1. Two view fields within the vascularized area of each specimen were then randomly selected and photomicrographed (0.54 mm^2 per view field) and the vascular density (VD) was estimated from these photomicrographs by counting the number of intersections between a bar patterned lattice and the vessels (Norrby *et al.* 1990a); VD was the mean of the two view fields per window.

All the counting and measuring in sections and spreads was done blindly by one of us (JS).

Histamine release as a measure of mast-cell secretion

The histamine-receptor antagonists were administered s.c. for 4 consecutive days 1 hour before the animals received an i.p. injection of compound 48/80, i.e. at 0700 and 1400 h. The animals were killed on the 5th day and three randomly selected, medium-sized mesenteric windows were excised from each animal, pooled, and immediately frozen and stored at -70°C. Before analysis, the tissue was homogenized with 0.4 M $HClO_4$, to which 0.002% ascorbic acid and 0.2% EDTA were added.

Histamine was quantified by a fluorometric OPT-reaction (Enerbäck & Wingren 1980) and the total protein content was quantified according to Lowry *et al.* (1951); the histamine content was expressed as ng histamine base per μg protein.

Statistics

The difference between treatment groups was assessed using the Mann–Whitney *U*-rank sum test (two-tailed) for unpaired observations. A *P*-value of $\leqslant 0.05$ was considered significant. For No/UL, VA and VD the means of four windows per animal were used as independent data. As a result, there were eight independent data per variable and treatment group.

Results

There was no significant difference in body weight between the animals treated with a histamine-receptor antagonist as compared with the saline-treated controls (data not shown) and no side-effects from the histamine-receptor blocking agents were observed. Neither of the histamine receptor antagonists at the middle dose significantly affected the 48/80-induced mast-cell secretion in the test tissue (Table 1).

The effect of histamine-receptor antagonists on the number of vessel profiles per unit tissue length (No/UL)

In the first experiment, where low doses of the histamine-receptor antagonists were used, No/UL was 0.931 ± 0.137 (mean ± s.e.m.) in controls. Both receptor antagonists produced a prominent angiostatic effect: on average No/UL was reduced by 40.3% ($P \leqslant 0.07$) by brompheniramine maleate at a dose of 0.150 mg/kg b.w. and by 53.0% ($P \leqslant 0.01$) by metiamide at a dose of 2 mg/kg b.w.,

Table 1. Histamine concentration in mesenteric windows in rats co-treated twice daily for 4 days with saline, brompheniramine maleate (BPA) or metiamide s.c. and compound 48/80 i.p. No significant difference in statistical terms was found between any two groups. Mean ± s.e.m.

Treatment	Dose s.c. (mg/kg)	Histamine concentration (ng/μg protein)
Saline (12)	—	1.031 ± 0.037
BPA (10)	0.225	1.113 ± 0.041
Metiamide (10)	3.000	1.094 ± 0.071

Figures in parentheses indicate number of animals.

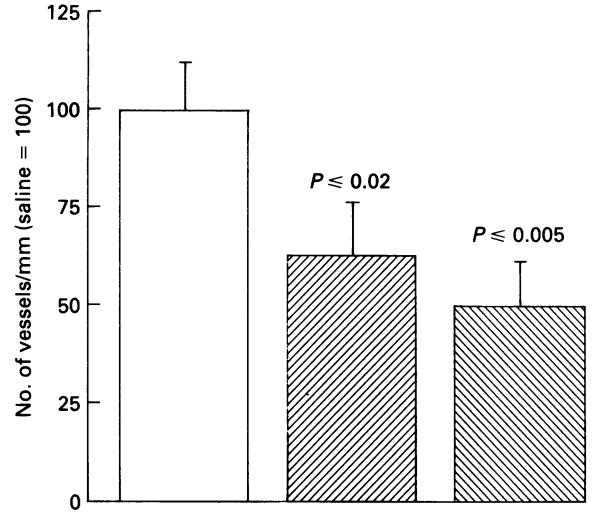

Figure 2. The systemic effect of ■, the s.c. administered histamine-1 receptor antagonist brompheniramine maleate (BPA) and ▧, the s.c. administered histamine-2 receptor antagonist metiamide on mast-cell mediated angiogenesis; □, controls received saline s.c. The number of vessel profiles per mm tissue was normalized by setting the mean of the saline treated controls at 100 in each experiment and then expressing every individual value in the same experiment as a percentage of this mean. Each group comprised 20 animals. Mean ± s.e.m.

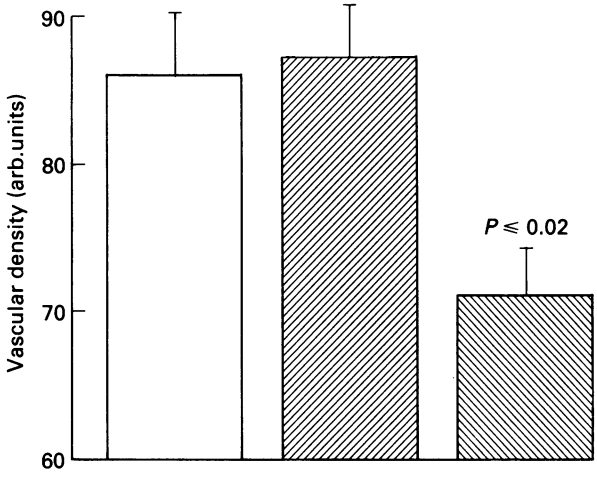

Figure 3. The systemic effect of ■, the H_1-receptor blocker brompheniramine maleate (BPA) and ▧, the H_2-receptor blocker metiamide on vascular density (VD) in mast-cell mediated angiogenesis. The H_2-receptor antagonist significantly decreased the vascular density compared with □, the saline-treated animals ($P \leqslant 0.02$) and with the BPA-treated animals ($P \leqslant 0.02$), whereas the histamine-1 receptor blocker had no significant effect as compared with the saline-treated controls. There were eight animals in each group. Mean ± s.e.m.

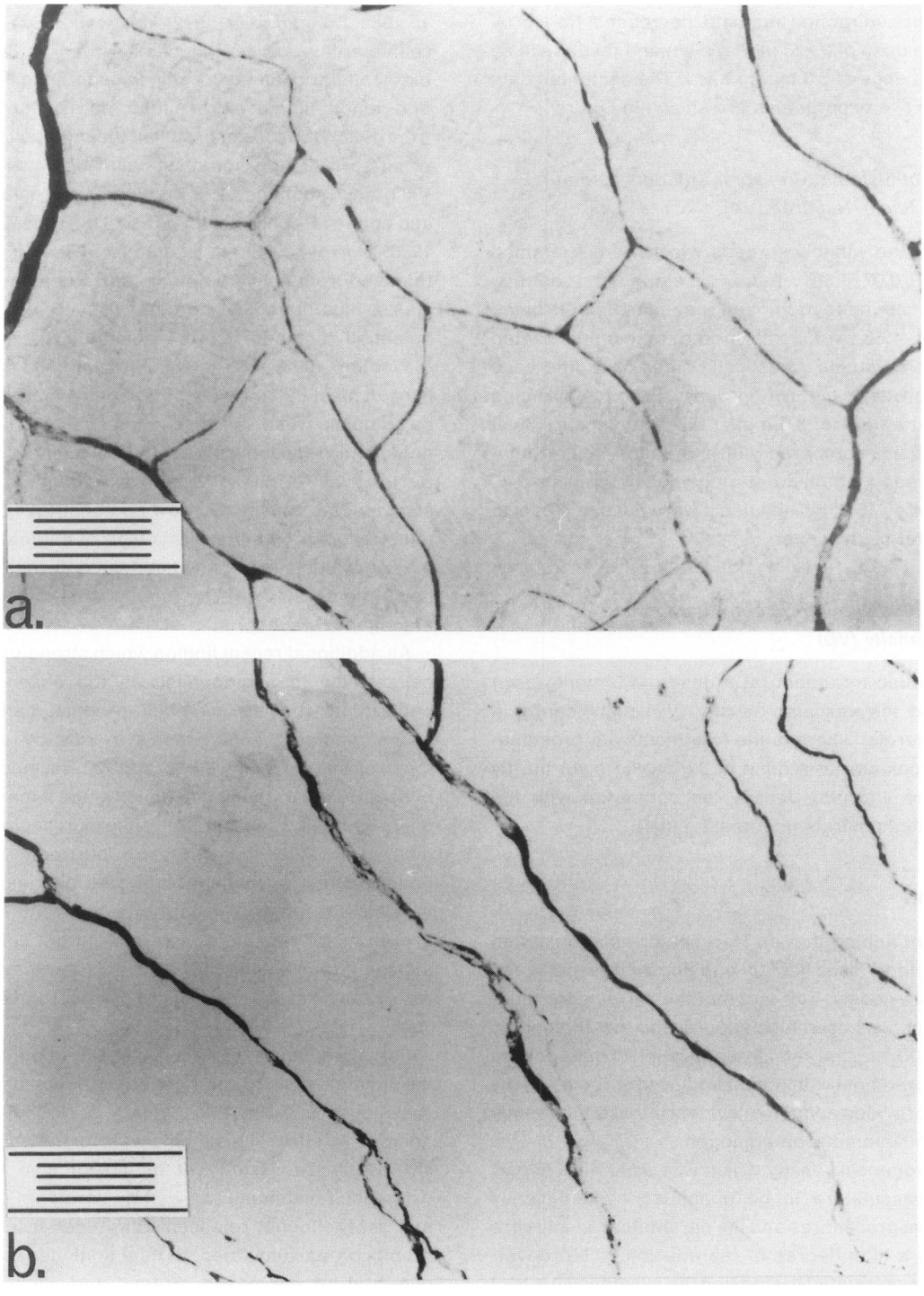

Figure 4. Photomicrographs within the vascularized area of spreads of intact mesenteric windows from animals receiving s.c. injections of a, saline and b, metiamide. The number of intersections between the bar-patterned lattice used (not shown) and the vessels was 83 in a and 68 in b. These values are close to the mean for the vascular density in each group (see Figure 3). Controls (a) showed many microvascular branches, whereas specimens from the metiamide treated animals showed an increased distance between branching points, apparently due to the suppression of branching. The vessels were visualized by an i.v. infusion of indian ink. Inserted micrometres: 10 μm between lines. Clearly, many vessels had a diameter about 5 μm.

compared with controls. In the subsequent experiment, where No/UL was 0.834 ± 0.168 (mean ± s.e.m.) in controls, brompheniramine maleate decreased No/UL by 33.5% (at a dose of 0.225 mg/kg b.w.) and metiamide by 47.1% (at a dose of 3.0 mg/kg b.w.). The combined data from these two experiments are shown in Figure 2.

The effect of histamine-receptor antagonists on the relative vascularized area (VA)

The size of the window spreads which were examined was 1.64 ± 0.07 cm^2 (mean ± s.e.m.) in controls, 1.68 ± 0.06 cm^2 in the brompheniramine maleate-treated group and 1.56 ± 0.06 cm^2 in the metiamide-treated group. There was no statistically significant difference in size between any two groups. Brompheniramine maleate (at a dose of 3.0 mg/kg b.w.) reduced VA by a mean of 20.2% compared with the saline-treated controls, whereas metiamide at a dose of 75.0 mg/kg b.w. reduced VA by 15.5%. The effects were not significant in statistical terms, however.

The effect of histamine-receptor antagonists on the vascular density (VD)

The metiamide treatment (at a dose of 75 mg/kg b.w.) suppressed the vascular density (VD) significantly in statistical terms, whereas the treatment with brompheniramine maleate (at a dose of 3.0 mg/kg b.w.) hardly affected the vascular density, as compared with the saline-treated controls (Figures 3 and 4).

Discussion

The present finding that an H_2-receptor antagonist can inhibit angiogenesis has to our knowledge not been reported previously. The fact that the H_1-receptor antagonist which was used also suppressed the angiogenic reaction following the selective activation of mast cells *in situ* is in agreement with recent findings (Sörbo & Norrby 1992) and provides additional evidence that endogenous mast-cell histamine is an angiogen.

The angiogenesis assay which was used here allows the microvasculature to be quantified by technically independent procedures and the parameters which were used show a high degree of reproducibility. Moreover, the assay is well suited to studying the systemic effects of drugs on *de novo* angiogenesis in normally vascularized mammalian tissue (Norrby *et al.* 1990b; Norrby & Sörbo 1992; Sörbo & Norrby 1992). Using this assay, the occurrence of mast-cell mediated angiogenesis was reported for the first time (Norrby *et al.* 1986). Mast-cell mediated stimulation of ongoing organogenic angiogenesis in the CAM was subsequently reported (Clinton *et al.* 1988; Duncan *et al.* 1992). It is well known that mast-cell secretion *in vivo* induces a variety of cellular and extracellular changes locally, including the degradation and remodelling of extracellular matrix (Norrby & Enestrom 1984; Dabbous *et al.* 1986), the metabolic activation of all surrounding non-mast-cells (Norrby & Enestrom 1984) and mitogenesis in many adjacent mesenchymal and epithelial cells (Norrby *et al.* 1976; Franzén & Norrby 1980; Norrby 1983). It is also known that mediators released from activated mast cells can stimulate fibroblasts, macrophages and other tissue cells to secrete potentially angiogenic proteases and cytokines (Norrby & Woolley 1993). The molecular and cellular mechanisms of mast-cell mediated angiogenesis may therefore be complex, which is also suggested by the fact that the angiogenic reaction lasts for several months (Jakobsson & Norrby 1991), long after the initiating angiogenic stimulus has ceased. By now demonstrating that histamine receptors suppress this type of angiogenesis, we may conclude that the mast-cell not only initiates but, at least in part, also directly regulates the outcome of the angiogenic reaction.

An additional recent finding which strongly links mast-cell activity to angiogenesis is the observation that heparin, a specific mast-cell product, can augment saline mediated angiogenesis in rats by a systemic mode of action (Norrby & Sörbo 1992). The mode of action of heparin appears to be complex since it may affect not only endothelial cells through specific receptors but also the extracellular concentration of freely accessible heparin-binding growth factors, their biological activity, their ligand function in terms of high and low-affinity growth factor receptors of various target cells and the molecular composition and function of the extracellular matrix, as discussed elsewhere (Klagsbrun 1992; Norrby 1993).

For many years now, reports have been published linking mast-cells to angiogenesis, as recently reviewed (Meininger & Zetter 1992; Norrby & Woolley 1993). An angiogenic effect of histamine was, for instance, demonstrated by Zauberman *et al.* (1969) who found that exogenous histamine (as well as serotonin, bradykinin and acetylcholine) released from tubes inserted in the rabbit cornea stimulated corneal angiogenesis in about one-third of the cases. Fraser and Simpson (1983) reported that histamine (dissolved in saline at 10^{-6} M) when applied as a liquid drop stimulated the ongoing onganogenic angiogenesis locally in the chick chorioallantoic membrane (CAM), the angiogenic response being judged by 'a qualitative and subjective method'.

Subsequently, Thompson and Brown (1987) claimed that a sublethal dose of exogenous histamine (at a concentration of 0.09 M) was required to convincingly increase the vascularity of the CAM, when quantified in the lengths and number of the fourth order of arterial branches and in vessels counted in histological cross-sections. Duncan *et al.* (1992) recently demonstrated that compound 48/80 administered to the CAM resulted in a pattern of DNA synthesis that resembles a composite of effects of histamine and heparin, although this variable is not obviously specific to the vascular component in angiogenesis.

In this context it should be remembered that angiogenesis involves the activity not only of endothelial cells but also of many, if not all, other adjacent tissue-bound cells (Shing *et al.* 1985; Rhodin & Fujita 1989; Nehls *et al.* 1992). In normal growth, regeneration, repair and tumour growth in which angiogenesis occurs, the tissue cells of the host as well as the tumour cells have an increased metabolic need, but the nutrient delivery and waste removal are limited as the diffusion distances may be too long until new vessels have developed (Adair *et al.* 1990). So, increased DNA synthesis and the proliferative activity of these non-endothelial cells (Duncan *et al.* 1992), as well as their capacity to produce angiogenic factors (Folkman 1992), seem to be inherent parts of angiogenesis. It may thus be pertinent to mention that the mast-cell mediated mitogenesis of fibroblasts and mesothelial cells, which are the predominant cell types in the rat mesenteric window (Norrby & Enestrom 1984), is markedly suppressed, *in vivo* and in organ culture, by the H_2-receptor antagonist metiamide used in the present study (Norrby 1980; 1985). Furthermore, exogenous histamine at a concentration of approximately 10^{-10} M is mitogenic not only to cultured 3T3 cells (Norrby 1973) but also to the rat mesenteric window fibroblasts and mesothelial cells in organ cultured intact mesenteric windows (Franzén & Norrby 1980). In agreement with these findings, the histamine induced proliferation of normal adult human lung fibroblasts was entirely abrogated by exposing the cells to an H_2-receptor antagonist (Jordana *et al.* 1988). Mast-cell granule histamine is also reported to be mitogenic to cultured human microvascular endothelial cells, the effect being reproduced by an H_1-agonist and inhibited by an H_1-antagonist (Marks *et al.* 1986). Clearly, endogenously released mast-cell histamine in the present study exerted its effects through both H_1 and H_2-receptors of as yet unidentified target cells. It is however known that endothelial cells carry both these types of histamine receptor (Smaje *et al.* 1988; Nakamura *et al.* 1988).

In the present study, the H_1-receptor antagonist brompheniramine maleate inhibited No/UL significantly but suppressed VA only slightly and VD hardly at all, whereas the H_1-receptor blocker clemastine in a previous study (Sörbo & Norrby 1992) suppressed VA significantly and VD marginally (No/UL was not then determined). This somewhat different effect on the neovascularization pattern of the two H_1-receptor antagonists may, hypothetically, be related to differences in drug activity and dose. Since the recorded inhibition by histamine-receptor blockade varied from 20 to 50% it is likely that other preformed or newly generated mast-cell mediators also are angiogenic.

In conclusion, by using histamine H_1 and H_2-receptor antagonists systemically at apparently non-toxic doses, we found that histamine released endogenously from connective tissue mast-cells in their genuine microenvironment stimulates angiogenesis. The fact that the occupancy of H_2-receptors can cause angiogenesis is reported here for the first time.

Acknowledgements

The study was supported by grants from the Swedish Medical Research Council, project no. 5942, and the Göteborg Medical Society. We thank Mrs Gunvor Jefferth and Miss Ann Nehlmark for their skilful technical assistance.

References

ADAIR T.H., WILLIAM J.G. & MONTANI J.P. (1990) Growth regulation of the vascular system: evidence for a metabolic hypothesis. *Am. J. Physiol.* **259,** 393–404.

BLACK J.W., DUNCAN W.A.M., EMETT J.C., GANELLIN C.R., HESSELBO T., PARSONS M.E. & WYLLIE J.H. (1973) Metiamide—an orally active histamine H_2-receptor antagonist. *Agents Actions* **3/3,** 133–137.

CLINTON M., LONG W.F., WILLIAMSON F.B., DUNCAN J.I. & THOMPSON W.D. (1988) Effect of the mast cell activator compound 48/80 and heparin on angiogenesis in the chick chorioallantoic membrane. *Int. J. Microcirc. Clin. Exp.* **7,** 315–326.

COWEN T., TRIGG P. & EADY R.A.J. (1979) Distribution of mast cells in human dermis: development of a mapping technique. *Br. J. Dermatol.* **100,** 635–640.

DABBOUS M.K., WOOLEY D.E., HANEY L., CARTER L.M. & NICOLSON G.L. (1986) Host-mediated effectors of tumor invasion: role of mast cells in matrix degradation. *Clin. Exp. Metast.* **4,** 141–152.

DUNCAN J.I., BROWN F.I., MCKINNON A., LONG W.F., WILLIAMSON F.B. & THOMPSON W.D. (1992) Patterns of angiogenic response to mast cell granule constituents. *Int. J. Microcirc. Clin. Exp.* **11,** 21–33.

ENERBÄCK L., FRANZÉN L. & NORRBY K. (1976) A tissue model for the study of cell proliferation parameters *in vivo*. *Histochemistry* **47,** 207–218.

ENERBÄCK L. & NORRBY K. (1989) The mast cells. In *Cell Kinetics of*

the Inflammatory Reaction. Ed. O.H. Iversen. *Current Topics in Pathology*. Volume 79. Berlin: Springer. pp. 169–204.

ENERBÄCK L. & WINGREN U. (1980) Histamine content of peritoneal and tissue mast cells of growing rats. *Histochemistry* **66,** 113–124.

FOLKMAN J. (1990) What is the evidence that tumors are angiogenesis dependent? *J. Natl Cancer Inst.* **82,** 4–6.

FOLKMAN J. (1992) Angiogenesis—retrospect and outlook. In *Angiogenesis: Key principles—Science—Technology—Medicine*. Eds R. Steiner., P.B. Weisz & R. Langer. Basel: Birkhäuser. pp. 4–13.

FRANZÉN L. & NORRBY K. (1980) Local mitogenic effect of tissue mast cell secretion. *Cell. Tissue Kinet.* **13,** 635–642.

FRASER R.A. & SIMPSON J.G. (1983) Role of mast cells in experimental tumour angiogenesis. In *Development of the Vascular System*. CIBA Foundation Symposium 100. Eds J. Nugent & M. O'Connor. London: Pitman. pp. 120–131.

GARRISON J. (1990) Histamine, bradykinin, 5-hydroxytryptamine, and their antagonists. In *Goodman and Gillman's: The Pharmacological Basis of Therapeutics*. New York: Maxwell Macmillan. pp. 575–599.

GORDON J.R., BURD P.R. & GALLI S.J. (1990) Mast cells as a source of multifunctional cytokines. *Immunol. Today* **11,** 458–464.

HOBSON B. & DENEKAMP J. (1984) Endothelial proliferation in tumours and normal tissues: continuous labelling studies. *Br. J. Cancer* **49,** 405–413.

JAKOBSSON A. & NORRBY K. (1991) Kinetics of a mammalian angiogenic response. *Cell Prolif.* **24,** 59.

JORDANA M., BEFUS D.A., NEWHOUSE M.T., BIENENSTOCK J. & GAULDIE J. (1988) Effect of histamine on proliferation of normal human adult lung fibroblasts. *Thorax* **43,** 552–558.

KLAGSBRUN M.A. (1992) Mediators of angiogenesis: the biological significance of basic fibroblast growth factor (bFGF)-heparin and heparan sulfate interactions. *Cancer Biol.* **3,** 81–87.

LOWRY O.H., ROSEBOROUGH N.J., FARR A.L. & RANDALL R.J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.

MARKS R.M., ROCHE W.R., CZERNIECKI M., PENNY R. & NELSON D.S. (1986) Mast cell granules cause proliferation of human microvascular endothelial cells. *Lab. Invest.* **55,** 289–294.

MEININGER C.J. & ZETTER B.R. (1992) Mast cells and angiogenesis. *Cancer Biology* **3,** 73–79.

MELLBLOM L. & ENERBÄCK L. (1979) Protein content, dry mass and chemical composition of individual mast cells related to body growth. *Histochemistry* **63,** 129–143.

NAKAMURA M., ODA M., KANEKO K., HONDA K., KOMATSU H. & TSUCHIA M. (1988) Radioautographic characterization of H1 and H2 receptor antagonist-binding sites in the rat gastric mucosal microcirculatory system. *Adv. Exp. Med. Biol.* **242,** 151–160.

NEHLS V., DENZER K. & DRENCKHAHN D. (1992) Pericyte involvement in capillary sprouting during angiogenesis in situ. *Cell Tissue Res.* **270,** 469–474.

NORRBY K. (1973) Effect of heparin, histamine and serotonin on the density-dependent inhibition of replication in two fibroblastic cell lines. *Virchow's Arch. (Cell Pathol.)* **15,** 75–93.

NORRBY K. (1980) Mast cell histamine, a local mitogen acting via H2 receptors in nearby tissue cells. *Virchow's Arch. (Cell Pathol.)* **34,** 13–20.

NORRBY K. (1983) Intradermal mast-cell secretion causing cutaneous mitogenesis. *Virchow's Arch. (Cell Pathol.)* **42,** 263–269.

NORRBY K. (1985) Evidence of mast-cell histamine being mitogenic in intact tissue. *Agents Actions* **16,** 287–290.

NORRBY K. (1993) Heparin and angiogenesis: a low-molecular-weight fraction inhibits and a high-molecular-weight fraction stimulates angiogenesis systemically. *Haemostasis* **23 (Suppl. 1),** 141–149.

NORRBY K., ENERBÄCK L. & FRANZEN L. (1976) Mast cell activation and tissue cell proliferation. *Cell Tissue Res.* **170,** 289–303.

NORRBY K. & ENESTRÖM S. (1984) Cellular and extracellular changes following mast-cell secretion in avascular rat mesentery. An electron-microscopic study. *Cell Tissue Res.* **235,** 339–345.

NORRBY K., JAKOBSSON A. & SÖRBO J. (1986) Mast-cell-mediated angiogenesis: a novel experimental model using the rat mesentery. *Virchow's Arch. (Cell Pathol.)* **52,** 195–206.

NORRBY K., JAKOBSSON A. & SÖRBO J. (1989) Mast-cell secretion and angiogenesis, a quantitative study in rats and mice. *Virchow's Arch. (Cell Pathol.)* **57,** 251–256.

NORRBY K., JAKOBSSON A. & SÖRBO J. (1990a) Quantitative angiogenesis in spreads of intact rat mesenteric windows. *Microvasc. Res.* **39,** 341–348.

NORRBY K., JAKOBSSON A. & SÖRBO J. (1990b) On mast-cell-mediated angiogenesis in rat mesenteric-window assay. *Agent Actions* **30,** 231–233.

NORRBY K., & SÖRBO J. (1992) Heparin enhances angiogenesis by a systemic mode of action. *Int. J. Exp. Pathol.* **73,** 147–155.

NORRBY K. & WOOLLEY D. (1993) Role of mast cells in mitogenesis and angiogenesis in normal tissue and tumour tissue. *Adv. Biosciences* **89,** 71–116.

RAKUSAN K., SARKAR K., TUREK Z. & WICKER P. (1990) Mast cells in the rat heart during normal growth and in cardiac hypertrophy. *Circ. Res.* **66,** 511–516.

RHODIN J.A. & FUJITA H. (1989) Capillary growth in the mesentery of normal young rats. Intravital video and electron microscope analyses. *J. Submicrosc. Cytol. Pathol.* **21,** 1–34.

SHING Y., FOLKMAN J., HAUDENSCHILD C., LUND D., CRUM R. & KLAGSBRUN M. (1985) Angiogenesis is stimulated by a tumor-derived endothelial growth factor. *J. Cell. Biochem.* **29,** 275–287.

SMAJE L.H., NOOR N.M. & CLOUGH G.F. (1988) Changing sensitivity to H1 and H2 receptor agonists in the growing vasculature. *Adv. Exp. Med. Biol.* **242,** 145–150.

SÖRBO J. & NORRBY K. (1992) Mast-cell histamine expands the microvasculature spatially. *Agents Actions*. S.C.I. C387–C389.

THOMPSON W.D. & BROWN F.I. (1987) Quantitation of histamine-induced angiogenesis in the chick chorioallantoic membrane: mode of action of histamine is indirect. *Int. J. Microcirc. Clin. Exp.* **6,** 343–357.

ZAUBERMAN H., MICHAELSSON I.C., BERGMAN F. & MAURICE D.M. (1969) Stimulation of neovascularization of the cornea by biogenic amines. *Exptl Eye Res.* **8,** 77–83.