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Stem-cell factor, the kit ligand, induces direct degranulation of rat peritoneal mast cells *in vitro* and *in vivo*: dependence of the *in vitro* effect on period of culture and comparisons of stem-cell factor with other mast cell-activating agents

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SUMMARY

We report that stem-cell factor (SCF), the ligand of the receptor encoded by the c-*kit* proto-oncogene, is a potent activator of degranulation of rat peritoneal mast cells *in vitro* and *in vivo*. Freshly isolated, purified mast cells were relatively unresponsive to SCF (4–500 ng/ml) but progressively acquired responsiveness to this agent, assessed as serotonin (5-HT) release, during 48 hr culture *in vitro*. The cells showed a similar kinetic pattern of acquisition of responsiveness to anti-IgE but responded fully to calcium ionophore A23187 or compound 48/80 regardless of time in culture. Acquisition of mast cell responsiveness to SCF or anti-IgE was not due to serum factors or to recovery from the Percoll purification procedure. During culture, mast cell expression of the SCF receptor (SCFR) increased, and this may explain in part the increased responsiveness to SCF. However, surface IgE expression remained constant, and the increased responses to anti-IgE therefore must reflect changes in components of the secretion-coupling pathway that are activated subsequent to IgE cross-linking. The unresponsiveness of freshly isolated peritoneal mast cells to SCF or anti-IgE does not reflect a state of *in vivo* unresponsiveness, as peritoneal mast cells degranulated *in vivo* in response to these agents. We conclude that in terms of their responsiveness to SCF or anti-IgE, cultured tissue mast cells may be more representative than freshly isolated mast cells of secretory function *in vivo*, and therefore may be more appropriate for physiological or pharmacological studies of SCF- or IgE-dependent secretory responses.

INTRODUCTION

Stem-cell factor (SCF), the product of the *Sl* gene in the mouse[1–4] is a growth factor that is important in the development of pluripotent haematopoietic stem cells, neural crest-derived melanocytes and erythroid and mast cell lineages (reviewed in refs 5,6). SCF is produced by several cell types including stromal cells in the bone marrow and fibroblasts in tissues; it is synthesized as a biologically active transmembrane protein and secreted as an active factor.[5,6] SCF exerts its diverse effects via interaction with its receptor (SCFR), a tyrosine kinase encoded by the c-*kit* proto-oncogene.[5,6]

SCF promotes the survival, proliferation and differentiation of mast cells *in vitro* and *in vivo* with effects documented in the mouse,[2,3,7–17] rat,[12,18,19] experimental primates[20] and humans.[21–25] In addition, SCF influences mast cell secretory function. For example, it enhances IgE-mediated degranulation of mast cells isolated from the mouse peritoneal cavity,[26] human skin[27] or human lung.[28] Direct intradermal injection of SCF into mice induces degranulation of skin mast cells *in vivo*.[29] Paradoxically, SCF has been reported not to be a potent mast cell secretagogue *in vitro*. At concentrations of up to 100 ng/ml, SCF induced serotonin (5-HT) or histamine release only weakly (< 15% net release) from mouse peritoneal mast cells[26] or human skin mast cells[27] and had little or no direct secretory effect when tested against human lung mast cells.[28] Also, rat peritoneal mast cells have been reported to be essentially unresponsive to any direct effects of SCF except in the presence of high concentrations of phosphatidylserine (e.g. 20 μg/ml), conditions that are unlikely to occur naturally.[30]

The reasons for the differences in the degranulating effects

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Abbreviations: cDMEM, complete Dulbecco's modified essential medium containing 5% FCS; CTMC, connective tissue-type mast cells; DNP–HSA, dinitrophenyl–human serum albumin; FcεRI, the high-affinity receptor for IgE; FCS, fetal calf serum; 5-HT, 5-hydroxytryptamine (serotonin); SCF, stem-cell factor; SCFR, stem-cell factor receptor, the product of the c-*kit* gene.

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of SCF seen *in vivo* as opposed to *in vitro* have not been defined. To address this problem we re-examined the capacity of SCF to induce directly exocytosis of isolated purified mast cells. In particular, we considered the possibility that the responsiveness of rat peritoneal mast cells to SCF, or to other secretagogues, may vary significantly according to the time these cells have been cultured *in vitro*.

Unexpectedly, we found that purified rat peritoneal mast cells became signficantly more responsive to the direct releasing activity of this cytokine, as well as to activation by anti-IgE, after a prolonged period of culture. By contrast, the period of time in culture had no significant effect on the responsiveness of rat peritoneal mast cells to activation by either compound 48/80 or the calcium ionophore A23187. These findings show that under certain culture conditions SCF is a potent direct activator of rat peritoneal mast cells.

MATERIALS AND METHODS

Cells, culture and challenge

Mast cells were obtained by peritoneal lavage of female Wistar rats (Liverpool departmental outbred stock, University of Liverpool, UK) with 100 ml of RPMI-1640 medium (Gibco Life Technologies, Paisley, UK). The cells were sedimented by centrifugation at 150 **g** for 8 min and the pellets were pooled in Dulbecco's modified essential medium (DMEM, Gibco) containing 5% heat-inactivated fetal calf serum (FCS; Sera-Lab, Crawley Down, UK) and 50 μg/ml gentamicin (complete DMEM; cDMEM). The cells were sedimented again and the pellet was resuspended in 7·5 ml of 72·5% isotonic Percoll (Sigma, Poole, UK), overlaid with 2 ml cDMEM, and centrifuged at 300 **g** for 8 min. Mast cells were recovered from the pellet, washed and resuspended in cDMEM. Mast cells comprised > 98% of total cells after Percoll fractionation, and 5–7% of total cells in unfractionated populations, as determined by metachromatic staining with 0·02% toluidine blue.

The cells were cultured at $4–6 \times 10^5$ mast cells/ml in 1·0-ml volumes of cDMEM in 15-ml conical plastic tubes at 37° in 5% CO_2 in air, for various time periods. In some experiments the cells were cultured in DMEM containing 0·1% Albumax (lipid-rich albumin; Gibco) in place of serum.

At the termination of cultures the cells were sedimented, washed twice and suspended in 1–2 ml of cDMEM. The cells (150 μl) were added to 150 μl of challenge medium (as control) or 150 μl of cDMEM containing various concentrations of recombinant rat SCF (*Escherichia coli*-derived rrSCF^{164}; Amgen, Thousand Oaks, CA), sheep anti-rat IgE (ICN Biomedicals Limited, Thame, UK), compound 48/80 (Sigma) or calcium ionophore A23187 (Sigma), or, to lyse the cells, 0·05% Triton-X-100 (Sigma). The cells were then incubated for 30 min at 37° in 5% CO_2 in air, after which they were centrifuged at 150 **g** for 3 min and 150 μl of supernatant fraction removed for 5-HT release assay.[26] In experiments to measure histamine release the challenge volume was 1·0 ml. Histamine in the supernatant fraction (0·9 ml) was assayed by the *o*-phthalaldehyde method after the full extraction procedure.[31] Percentage specific release of 5-HT or histamine was calculated as: $[(a - b)/c)] \times 100$, where a is the amount of mediator released from stimulated cells, b is that released from unstimulated cells and c is total cellular content. Background release of 5-HT or histamine from unstimulated cells was always 1–3% of the cell-associated total.

Flow cytometric analysis of mast cell-surface SCFR and IgE

Expression of cell-surface SCFR and bound IgE was analysed by flow cytometry of purified mast cells cultured for 1 or 24 hr. Purified mast cells (10^5) were washed with ice-cold phosphate-buffered saline PBS (1 ml) and sedimented (6500 r.p.m. for 1 min in an angled microfuge). The supernatant PBS was discarded and the cell pellet resuspended in 50 μl of ice-cold PBS. For analysis of SCFR expression, 10 μl of biotin-labelled SCF (see below) was added to the cells for 30 min on ice. The cells were washed in ice-cold PBS (1 ml) and centrifuged, the supernatant discarded and 1 μl of streptavidin–phycoerythrin (Sigma) added to the cells in 50 μl PBS for 30 min on ice in the dark. The cells were washed and resuspended in 0·5 ml PBS on ice for flow cytometry. As the negative control, the biotinylated SCF was omitted. *Escherichia coli*-derived rrSCF^{164} (Amgen) was biotinylated by addition of 4 μl of a 0·03 M solution of *N*-hydroxysuccinimidobiotin (Sigma) to 100 μl of a 1 mg/ml solution of SCF; the mixture was allowed to react for 4 hr at room temperature. The solution was then diluted twofold with PBS and dialysed against 5000 vol of PBS at 4° overnight. The sample was then diluted with an equal volume of glycerol and frozen at −20°. The biotinylated SCF retained biological activity, as demonstrated by its undiminished capacity to induce 5-HT release from mast cells.

For analysis of cell-surface IgE expression, 1 μl of mouse monoclonal IgG1 fluorescein isothiocyanate (FITC)–anti-rat IgE (Serotec, Oxford, UK) was added to 50 μl of cells (10^5) for 30 min on ice. As the negative control, 1 μl of mouse monoclonal IgG1 FITC–anti-human CD4 (Serotec) was added to the cells. The cells were then washed and resuspended in 0·5 ml PBS on ice for flow cytometry.

Expression of SCFR or IgE was analysed using an EPICS-XL flow cytometer and associated software (Coulter Electronics, Luton, UK). Cell populations were gated for large granular cells on the basis of forward and 90° scatter. For all labelling the fluorescence threshold was set to give 1–2% positive cells after the negative control procedure. The percentage of positive cells for each test sample was then calculated as that proportion of the population which registered above the threshold level. Mean log fluorescence and peak fluorescence were calculated in arbitrary units; 10 000 events were analysed for each sample.

Mast cell activation in vivo

Female Wistar rats (250 g) received SCF (0·6 or 6 μg), anti-IgE (1 : 170 or 1 : 17), or compound 48/80 (3 or 30 μg) each in a volume of 1·0 ml sterile 0·9% NaCl, or 1·0 ml of 0·9% NaCl alone, by single intraperitoneal (i.p.) injection. The doses of these agents were selected on the basis of their activity *in vitro*. The same concentrations of SCF or anti-IgE induced substantial 5-HT release from 24-hr cultured but not from 1-hr cultured mast cells *in vitro*, whereas these concentrations of compound 48/80 induced 5-HT release from either 1- or 24-hr cultured mast cells (see the Results). The rats were killed by asphyxiation in CO_2 followed by cervical dislocation, and the peritoneal cells were recovered by lavage of the peritoneal cavity with 100 ml of RPMI-1640 medium. The cells were sedimented by centrifugation, washed and resuspended in

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3·0 ml of DMEM. Cells were maintained on ice throughout. Cytospins of the cells were prepared, fixed in methanol and then stained in 5% Giemsa for 20 min. The numbers of fully degranulated and total mast cells were counted by light microscopy by an observer who was unaware of the identity of the individual slides. The remaining cell suspension (2 ml) was removed and added to 2 ml of 0·8 N perchloric acid to precipitate proteins and to release and acidify cellular histamine. Histamine was then assayed by the *o*-phthalaldehyde method after the full extraction procedure.[31]

RESULTS

Responses of mast cells to SCF or other agents during culture

As assessed by release of 5-HT, culture of rat mast cells for 1, 6, 10, 24 or 48 hr led to a time-dependent increase in their responsiveness to challenge with either SCF (100 ng/ml) or anti-IgE (1/1000) (Fig. 1). Freshly isolated (1-hr cultured) mast cells were essentially unresponsive to either SCF or anti-IgE (< 5% net 5-HT release). A significant increase in responsiveness to anti-IgE was seen by 10 hr and to both agonists by 24 hr (Fig. 1). By 48 hr each agent induced 30–40% net release of 5-HT (Fig. 1). A similar time-dependent increase in mast cell responsiveness to SCF or anti-IgE was seen when degranulation was measured as release of histamine rather than of 5-HT (data not shown).

To test whether the relative unresponsiveness of the freshly isolated purified mast cells to SCF or anti-IgE might be an artefact of the Percoll purification procedure, we compared mast cells in unfractionated peritoneal cell populations with mast cells purified from the same starting populations. As shown in Table 1, both cell populations were initially unresponsive and both acquired responsiveness to SCF (100 ng/ml) or anti-IgE (1 : 1000) to a similar extent after culture for 24 hr.

The acquisition of responsiveness of purified mast cells to SCF or anti-IgE was not attributable to factors in serum, since similar enhancement of responsiveness after 24 hr was seen when cells were cultured in DMEM containing 0·1% Albumax in place of 5% FCS (Table 2).

Figure 2 shows the results of experiments looking at

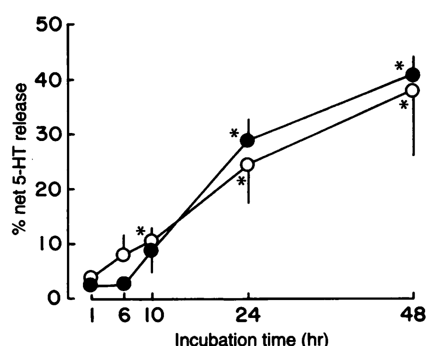

Figure 1. Effect of culture period (incubation time) on specific (net) release of 5-HT induced by SCF (●) or anti-IgE (○). Purified mast cells were cultured for various times, then washed and challenged for 30 min with SCF (100 ng/ml) or anti-IgE (1 : 1000). Results are means ± SEM for four experiments. (*$P < 0.05$ by two-tailed Mann-Whitney *U*-test by comparison with the 1-hr time-point).

concentration–response relationships for SCF, anti-IgE, 48/80 or calcium ionophore after 1 or 24 hr of culture. It is clear that 1-hr cultured mast cells were relatively unresponsive to SCF (4–500 ng/ml) or anti-IgE (1 : 10000–1 : 100) but released substantial proportions of 5-HT in response to 48/80 (0·1–2·5 μg/ml) or ionophore (0·2–0·8 μM). After 24-hr culture, responsiveness to SCF or anti-IgE increased dramatically while responsiveness to 48/80 or ionophore was unchanged. After 24-hr culture, mast cells released 15–35% net 5-HT at 20–500 ng/ml SCF and 16–24% net 5-HT at 1 : 1000–1 : 100 anti-IgE (Fig. 2).

Surface expression of SCFR or IgE on 1-hr and 24-hr cultured mast cells

Culture of purified rat mast cells for 24 hr led to increased cell-surface expression of SCFR compared to freshly isolated (1-hr cultured) mast cells (Fig. 3b,c) but no changes in levels of cell-surface IgE (Fig. 3e,f). Negative control fluorescence profiles are shown in Figs 3a,d for SCFR and IgE, respectively. Over four independent experiments, the proportion of mast cells that was detectably positive for SCFR increased significantly from approximately 30% to 75% between 1 and 24 hr of culture,

Table 1. Comparison of SCF- or anti-IgE-induced 5-HT release from unfractionated or purified rat peritoneal mast cells cultured for 1 or 24 hr

Stimulus	Cells	% net 5-HT release	
		1 hr	24 hr
100 ng/ml SCF	Purified mast cells†	1·6 ± 0·6	42·6 ± 7·6*
100 ng/ml SCF	Unfractionated peritoneal cells‡	2·1 ± 0·6	32·0 ± 10·9*
1 : 1000 anti-IgE	Purified mast cells	5·4 ± 2·2	39·0 ± 7·8*
1 : 1000 anti-IgE	Unfractionated peritoneal cells	5·8 ± 2·5	39·5 ± 11·2*

Results are means ± SEM for five independent experiments (*$P < 0.05$ for comparison of 24-hr cultured cells with 1-hr cultured cells by two-tailed paired Student's *t*-test).

† These preparations contained > 98% mast cells.

‡ These preparations contained ~7% mast cells.

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Table 2. Comparison of SCF- or anti-IgE-induced 5-HT release from purified peritoneal mast cells cultured for 1 or 24 hr in DMEM + 5% FCS or DMEM + 0·1% Albumax

Stimulus	Culture medium	% net 5-HT release	
		1 hr	24 hr
100 ng/ml SCF	DMEM + 5% FCS	0·7 ± 0·29	21·8 ± 2·4*
100 ng/ml SCF	DMEM + 0·1% Albumax	0·6 ± 0·1	13·1 ± 2·1*
1 : 100 anti-IgE	DMEM + 5% FCS	7·1 ± 2·0	36·3 ± 4·3*
1 : 100 anti-IgE	DMEM + 0·1% Albumax	6·0 ± 1·5	28·7 ± 4·4*

Results are means ± SEM for four experiments (*$P < 0.05$ for comparison of 24-hr cultured cells with 1-hr cultured cells by two-tailed paired Student's *t*-test).

while the proportion of detectably IgE positive cells did not change during culture (Table 3).

Activation of mast cells *in vivo*

Intraperitoneal administration of SCF (0·6 or 6 μg/rat) or anti-IgE (1 ml of 1 : 170 or 1 : 17/rat) produced a significant degree of mast cell degranulation *in vivo* (Table 4). Compound 48/80 at 30 μg/rat induced a marked decline in numbers of identifiable peritoneal mast cells, making it impossible to estimate the percentage of degranulated mast cells, whereas at 3 μg/rat mast cell numbers were reduced but a significant degree of degranulation was seen (Table 4). These changes in mast cell number and degranulation status were reflected by changes in the total histamine content of the peritoneal cells. SCF, anti-IgE or compound 48/80, each at the lower concentration tested, decreased peritoneal cell histamine content to 46%, 31% or 16% of control values, respectively (Table 4).

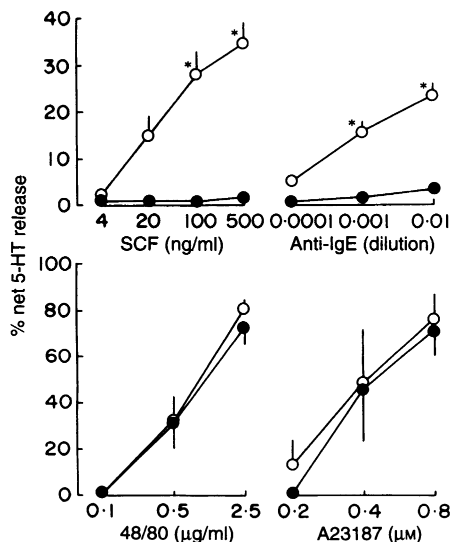

Figure 2. Concentration–response curves showing specific (net) release of 5-HT from purified mast cells challenged with SCF, anti-IgE, compound 48/80 or calcium ionophore A23187 after 1 hr (●) or 24 hr (○) of culture. Results are means ± SEM for four or five experiments (*$P < 0.05$ for comparison of 1-hr versus 24-hr cultured cells by the paired two-tailed Student's *t*-test).

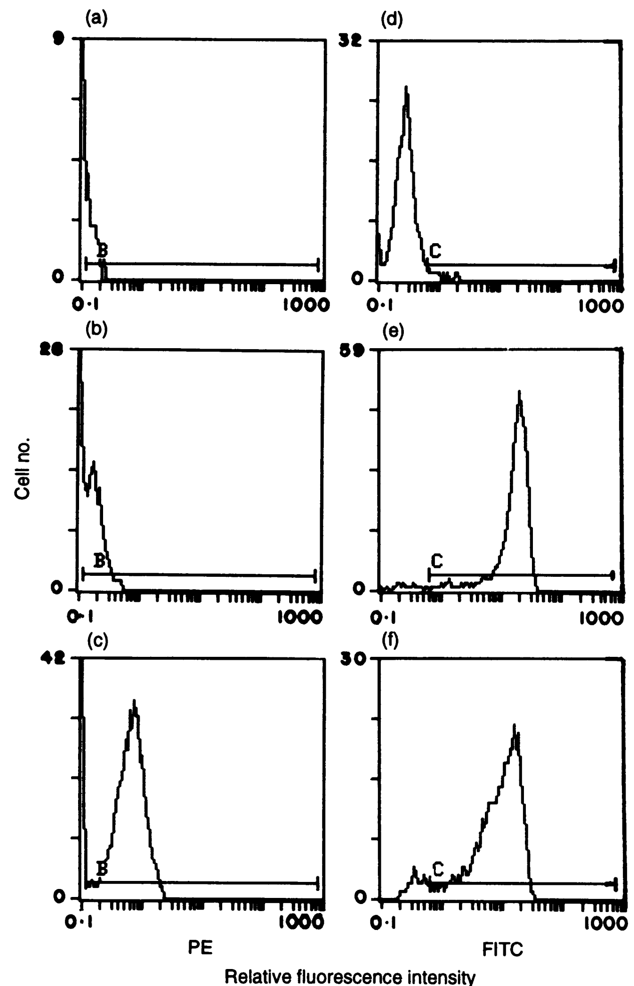

Figure 3. Flow cytometric analysis of expression of SCFR (left hand panels) and IgE (right hand panels) on purified mast cells. (a) negative control for SCFR staining; (b) SCFR expression after 1 hr of culture; (c) SCFR expression after 24 hr culture; (d) negative control for IgE expression; (e) IgE expression after 1 hr culture; (f) IgE expression after 24-hr culture. PE, phycoerythrin.

DISCUSSION

We found that freshly isolated rat peritoneal mast cells are unresponsive to SCF and relatively unresponsive to anti-IgE, but progressively acquire enhanced secretory responsiveness to these agonists during culture. After 1 hr of culture, release of 5-HT in response to SCF or anti-IgE was negligible; even after 10 hr of culture, release of 5-HT in response to either agent was < 40% of that seen after 24 hr of culture and < 30% of that seen at 48 hr. The increased responsiveness of cultured mast cells to SCF or anti-IgE was not dependent on the presence of exogenous factors *in vitro*: no cytokines were added to cultures and the same effect was seen regardless of whether the cells were cultured in FCS or in serum-free medium. Furthermore, it was not necessary to add phosphatidyl serine to the cells to observe responses to SCF, as had been reported previously.[30] We also investigated whether the acquisition of responsiveness might reflect recovery of cell function after the purification procedure. However, this was not the case, since unfractionated peritoneal mast cells and mast cells purified from the same starting

Table 3. Cell-surface expression of SCFR and bound IgE on purified rat peritoneal mast cells cultured for 1 or 24 hr

	SCFR		IgE	
	1 hr	24 hr	1 hr	24 hr
Net % positive cells	29·8 ± 7·5	74·6 ± 5·3*	91·6 ± 1·9	84·1 ± 3·0
Mean fluorescence intensity	0·22 ± 0·02	0·59 ± 0·15	6·0 ± 3·0	3·8 ± 1·7
Peak fluorescence intensity	0·14 ± 0·01	0·47 ± 0·16	8·1 ± 4·5	6·5 ± 3·9

Results are means ± SEM for four experiments (*$P < 0.05$ for comparison of 24-hr cultured cells with 1-hr cultured cells by two-tailed paired Student's *t*-test). In the experiments shown, SCF- or anti-IgE-induced 5-HT release increased between 1 and 24 hr of culture to an extent similar to that shown in Fig. 2.

populations acquired enhanced responsiveness to SCF or anti-IgE to a comparable degree. Moreover, freshly isolated and purified mast cells exhibited similarly robust responses to compound 48/80 or the calcium ionophore A23187, thus demonstrating that the freshly isolated mast cells were not generally unresponsive to secretory stimuli.

The acquisition by mast cells of secretory responsiveness to SCF was accompanied by a significant increase in expression of the SCFR during 24 hr culture (from 30% to 75% of positive mast cells). However, the cells' level of expression of plasma membrane-bound IgE remained constant during 24 hr of culture (84–92% of positive mast cells), showing that the acquisition of enhanced responsiveness to anti-IgE certainly was not related to changes in their surface IgE expression. We conclude that the enhanced responsiveness of rat peritoneal mast cells to SCF may in part reflect increases in SCFR expression, whereas enhanced responsiveness to anti-IgE reflects exclusively elements of the secretory response that are engaged subsequent to IgE cross-linking.

However, cultured rat peritoneal mast cells did not acquire time-dependent enhanced responsiveness to all of the agents tested. While responsiveness to SCF or anti-IgE increased markedly with time, the responses to compound 48/80 or calcium ionophore A23187 were essentially unaltered. Calcium ionophores or compound 48/80 trigger secretion by receptor-independent processes: the former act to insert or mobilize cellular Ca^{2+} [32] whereas compound 48/80 is thought to activate G proteins directly.[33–35] This suggests that receptor-dependent (SCF or IgE-mediated) but not receptor-independent secretory mechanisms are up-regulated during mast cell culture. Accordingly, we propose that the effect of long-term culture on the mast cells' responsiveness to receptor-dependent activation probably involves elements of the secretory machinery which come into play prior to Ca^{2+} mobilization or the activation of compound 48/80-inducible G proteins.

We did not examine whether long-term culture of mast cells influences their responsiveness to SCF or anti-IgE by affecting the same or different elements of the cells' signal transduction machinery. However, several lines of evidence indicate that the intracellular signalling processes that are initiated via the SCFR

Table 4. Effects of i.p. administration of SCF, anti-IgE, compound 48/80 or saline (control) on peritoneal mast cell degranulation and histamine content of total peritoneal cells

Treatment	Percent degranulated mast cells	Histamine content/total peritoneal cells (μg)
Experimental series 1		
Saline (n = 11)	15·2 ± 4·4	21·4 ± 3·6
SCF, 6 μg (n = 11)	32·9 ± 4·6**	11·8 ± 1·3**
Anti-IgE, 1 : 17 (n = 5)	28·0 ± 7·6*	4·4 ± 0·7***
Compound 48/80, 30 μg (n = 5)	Insufficient mast cells	0·7 ± 0·2***
Experimental series 2		
Saline (n = 4)	5·5 ± 1·7	13·6 ± 1·4
SCF, 0·6 μg (n = 4)	13·0 ± 2·1*	6·2 ± 1·9*
Anti-IgE, 1 : 170 (n = 4)	10·75 ± 0·8*	4·2 ± 1·6*
Compound 48/80, 3 μg (n = 4)	14·0 ± 2·7*†	2·2 ± 1·0*

Each compound was injected i.p. and 45 min later peritoneal cells were lavaged for analysis of histamine content and microscopic evaluation of mast cell degranulation (see the Materials and Methods). Results are means ± SEM for the number of animals indicated.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ by the one-tailed Mann-Whitney *U*-test by comparison with the saline-injected control group.

† These preparations contained considerably fewer intact mast cells.

or the FcεRI exhibit more similarities than might initially have been predicted. Thus, even though the SCFR is a receptor tyrosine kinase while the IgE receptor is not, we have reported that treatment of mouse bone marrow-derived mast cells with either SCF or IgE and specific antigen leads to a virtually indistinguishable pattern of expression of the early response genes c-*fos*, c-*jun* and *jun*-B,[36] and also induces very similar patterns of phosphorylation and activation of mitogen-activated protein kinases (MAPK), 90 000 MW-S6 kinases, and pp70-S6 kinases.[37] In human cutaneous mast cells, stimulation via the SCFR or the FcεRI induces a very similar pattern of changes in levels of intracellular-free calcium.[27] In light of these findings, it is possible that the enhanced 5-HT release that is observed in rat peritoneal mast cells which have been cultured for 24 or 48 hr prior to challenge with anti-IgE or SCF reflects changes in elements that are common to both signalling pathways, although increased expression of SCFR may also contribute to increased responses to SCF.

We also investigated the responsiveness of mast cells to stimulation by SCF or anti-IgE *in vivo*. In particular, we wondered whether the 'unresponsiveness' of freshly isolated mast cells might simply reflect the level of responsiveness which is expressed by these cells *in situ*. To address this question, we injected rats intraperitoneally with doses of SCF or anti-IgE corresponding to concentrations that induced *in vitro* substantial 5-HT release from mast cells challenged after 24 hr but not 1 hr of culture. We found that SCF or anti-IgE at these doses induced significant peritoneal mast cell degranulation and loss of peritoneal cell histamine content *in vivo*. Compound 48/80, again administered at doses comparable to concentrations used *in vitro*, also induced a marked mast cell degranulation and loss of peritoneal cell histamine *in vivo*.

It is clear from the above that mast cells in the peritoneal cavity *in situ* can indeed degranulate readily in response to SCF or anti-IgE. By contrast, freshly isolated mast cells were essentially unresponsive when challenged with either agent. Hence, it appears that the unresponsiveness of freshly isolated peritoneal mast cells to SCF or anti-IgE does not reflect the cells' state of responsiveness to these stimuli *in vivo*. It is more likely, we feel, that isolation of the peritoneal mast cells from the tissue microenvironment removes them from factors that maintain their secretory responses to SCFR- or FcεRI-dependent activation *in vivo*. If this is indeed the case, then one would also have to postulate that the purified mast cells (and/or the rare 'contaminating' cells in these preparations) can generate these factors (or factors with similar effects) during culture *in vitro*.

In conclusion, we have shown that, after 24 hr of culture, rat peritoneal mast cells degranulated and released 5-HT in response to challenge with SCF at concentrations as low as 20 ng/ml, whereas the freshly isolated cells were essentially unresponsive to this cytokine. Rat peritoneal mast cells also exhibited a similar pattern of responsiveness to challenge with anti-IgE. In addition to providing further evidence for the similarity of the mast cells' responsiveness to secretory signals initiated via the SCFR or the FcεRI, our findings indicate that mast cell secretory function may be regulated by previously unsuspected factors. Investigation of the nature of the signals responsible for this regulation may provide insight into the control of mast cell function during allergic responses and in other settings. Our findings may also have implications for the testing of mast cell-targeted drugs *in vitro*, as our data suggest that the responsiveness of long-term cultured tissue mast cells may more closely resemble that of mast cells *in situ* than does the responsiveness of freshly isolated cells.

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