Some studies on mast cell heterogeneity in man and other species

by

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A thesis presented to the University of London in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Science

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1990

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To my late father, mother

and Wai Shing

ABSTRACT

SOME STUDIES ON MAST CELL HETEROGENEITY IN MAN AND OTHER SPECIES

BY WAI LEUNG LIU

The development of methods for the enzymic dispersion and isolation of free mast cells from a number of target tissues has yielded valuable information concerning the phenomenon of mast cell heterogeneity. Now, a rapidly increasing body of evidence firmly indicates that mast cells from different species and from different tissues within a given animal may exhibit marked variations in their pharmacological, histochemical and morphological properties. In the present study, the functional characteristics of mast cells isolated from a range of animals including man are compared. Next, heterogeneity within a single species is investigated both in rat and human mast cells, and finally, a study on human colonic mucosal mast cells from patients with inflammatory bowel diseases (IBD) is reported.

Rat serosal mast cells were most responsive to the histamine releasing action of polybasic compounds and neuropeptides. Hamster and mouse peritoneal mast cells showed lower reactivity, while tissue mast cells from the pig, guinea pig and man were essentially refractory to these agents. This pattern of differential responsiveness was also observed for the anti-allergic chromones disodium cromoglycate (DSCG) and nedocromil sodium.

Rat mast cells from different anatomical locations responded in a heterogeneous fashion to the actions of histamine liberators and anti-allergic compounds. In general, cells from the peritoneum released the greatest percentage of their total cellular histamine in response to secretagogues of both immunological and non-immunological nature and were also the most sensitive towards agents that inhibited histamine release. In contrast, tissue mast cells were less responsive than their peritoneal counterparts. The order of reactivity was typically: mesentery >lung >intestine.

This apparent heterogeneity, however, was less clearly defined in human mast cells. Indeed, apart from some subtle histochemical and functional differences, mast cells from the lung parenchyma, colonic mucosa, colonic submucosa/muscle, stomach mucosa and uterine myometrium were, in many respects, similar to each other. Finally, an increase in mast cell numbers was observed in the colonic mucosa of patients suffering from Crohn's disease and ulcerative colitis. However, this apparent increase was not associated with an enhancement of their reactivity.

ACKNOWLEDGEMENTS

I am sincerely indebted to my supervisor Dr. Frederick L. Pearce for his invaluable advice and continuous support throughout the course of this study, and to my many colleagues for their help and encouragement. I would also like to express my gratitude to all the staff at the Joint Animal House, University College London; the surgeons and their medical teams at the Middlesex Hospital, University College Hospital and Elizabeth Garrett Anderson Hospital for the supply of both animal and human materials.

I am deeply grateful to the Science and Engineering Research Council for the award of a research studentship and to the Department of Chemistry, University College London, for the use of their facilities.

Lastly, 1 would like to dedicate this thesis to my late father for his inspiration.

CONTENTS

CHAPTER ONE

INTRODUCTION

1.1 Historical aspects

The role of the mast cell in allergy and inflammation is currently the subject of intense research. However, recognition of this cell type goes back as far as the latter half of the nineteenth century. The first clear reported description of the mast cell was made by Paul Ehrlich in his doctoral thesis in 1878 [1] in which he described a deeply staining metachromatic cell coupled with an "overfed" appearance. Hence, Ehrlich named the cell "mastzelle" from the German word "masten" which means to fatten or to feed. Later, he also discovered the circulating equivalent of the mast cell, namely the peripheral blood basophil [2].

The association between the mast cell and a pathological condition was first reported by Unna in 1894 [3] who observed that the cutaneous lesions of urticaria pigmentosa consisted almost exclusively of mast cells.

The role of the mast cell in immediate hypersensitivity reactions is now well documented [4], but it was Portier and Richet, as far back as 1902, who first recognised this phenomenon [5]. They observed the development of anaphylaxis in dogs given sea-anemone toxin and found that some of the animals did not die after the injection. However, those which survived exhibited a dramatic reaction upon a subsequent injection of the toxin. Further work on anaphylaxis was carried out by Dale [6] and Schultz [7], and it was Dale and Laidlaw [8] in a classic series of experiments who put forward the idea that histamine might be one of the chief factors involved in anaphylactic reactions since direct injection of the amine into animals and isolated tissues produced similar effects. Later work by Webb [9] implicated mast cells in the anaphylactoid reaction (so called because it resembled anaphylaxis but did not require prior sensitization).

In 1938, Wilander [10] reported that anaphylaxis in the dog is accompanied by damage to the mast cells in the liver, which is the shock organ of this species and a major source of its heparin. Later work on isolated perfused dog livers indicated that not only heparin but also histamine was derived from this organ [11,12].

The speculation of the possible involvement of the mast cell in immediate hypersensitivity reactions was further suggested by Riley and West [13,14] who found that there was a good correlation between the histamine content and mast cell counts in a variety of tissues.

Another major advance in this field was made in the late 1960's by the work of the Ishizakas [15,16] who identified the serum antibody responsible for immediate hypersensitivity reactions as belonging to a new and unique class of immunoglobulin named immunoglobulin E (IgE), and showed that the mast cell has surface receptors for this antibody [17,18].

1.2 The role of the mast cell in health and disease

Mast cells are widely distributed throughout the human body and the bodies of other vertebrate species. They are found essentially in those areas which come into frequent contact with foreign substances, namely in association with nerves and blood vessels in the loose connective tissue of the bronchi, conjunctiva, skin, lung, ear, nose and in the gastrointestinal tract [19,20].

Mammalian mast cells are ovoid or irregularly elongated in connective tissues whereas in suspension they are round and have diameter of 10-30 μm [21-23]. The characteristic feature of the mast cell is the presence of many dense cytoplasmic granules which sometimes occupy the cytoplasm to such an extent as to obscure the nucleus [23]. In most mammals, each granule averages 0.2-0.4 μm in diameter. There is species specificity for granule morphology. In the rat, the granule is amorphous in character [22,24] while human mast cell granules are more heterogeneous in substructural pattern. Dvorak and co-workers have identified four basic granule patterns (scroll, crystal, particle, mixed) [22,25]. Within an individual mast cell, all granules may

have a uniform substructural pattern (e.g. scrolls), or alternatively, cells may contain a mixture of granules of the various substructural types.

Since its reported discovery over 100 years ago, the mast cell and its blood counterpart the basophil have been linked to a number of human disorders especially in the immunopathology of immediate hypersensitivity reactions. Indeed, histologic and biochemical studies suggest that mast cells and the products of their activation play a role in the pathogenesis of inflammatory conditions such as rheumatoid arthritis [26,27], inflammatory bowel diseases [28,29], pulmonary fibrosis [30,31], sarcoidosis [32,33] and coronary artery diseases [34-36].

The unique distribution of the mast cell also points towards a possible role in defence mechanisms with the elimination of helminthic parasites a prime example [37]. Indeed, there appears to be a correlation between IgE levels, mast cell hyperplasia and eosinophilia upon parasitic infestation.

The mast cell has been traditionally linked with immediate hypersensitivity reactions. However, there is some evidence that the cell may also be involved in the mediation of a previously unrecognised early component of delayed type hypersensitivity (DTH) [38-41].

1.3 Activation of the mast cell

1.3.1 Immunological activation

IgE-directed substances are the classical stimuli by which mast cells are activated in nature. This phenomenon commences when our body comes into contact with a foreign particle (allergen/antigen) which stimulates the B-lymphocytes to differentiate into IgE antibody secreting plasma cells and a population of B-memory cells. Allergen also interacts with T-lymphocytes to produce either suppressor T-cells which inhibit the production of IgE or to give rise to helper T-cells which augment the process [42,43].

When IgE is secreted into the circulation, it can bind to the high affinity IgE

receptors on mast cells through its Fc_ε portion leaving free the recognition site for allergen (Fab). At this point, the cells are said to be sensitized but with no apparent sign of degranulation. However, subsequent exposure to the same allergen results in the cross-linking of IgE molecules and the consequent release of both preformed and newly synthesized mediators of anaphylaxis [44,45].

Information concerning the high-affinity IgE receptor has mainly been derived from work on rat peritoneal mast cells and rat cultured basophilic leukaemia (RBL) cells [46,47]. These studies concluded the receptor to be a glycoprotein with a content of about 13 % carbohydrate. The relative molecular mass of the receptor is around 87,000 and it consists of three subunits (α 45,000; β 33,000; γ 9,000). The α, β and γ subunits are all further divided into α_1, α_2, β_1 and β_2 polypeptides, with the two γ chains being identical (fig 1.1)

As discussed above, cross-linkage of IgE molecules by specific allergens is the initial trigger for the activation of the mast cell. However, monovalent allergens have been shown to be incapable of inducing anaphylaxis [48] while bi- or polyvalent allergens were effective in causing the cell to release its bioactive mediators [49]. The importance of the above observations was further supported by the findings that release may be produced by incubation of cells with chemically dimerised IgE, anti-IgE (IgG antibody directed against the Fc_ε heavy chains of IgE), concanavalin A (a lectin which cross-links IgE by binding to sugar moieties in the Fc_ε region) and antireceptor antibody (IgG antibody prepared against purified Fc_ε receptors) [50-52].

1.3.2 Non-immunological activation

In addition to activating mast cells by IgE-directed ligands, there are a number of non-immunological agents of diverse chemical structures which can also initiate the release process. In general, these agents can be classified into two main categories based on their mode of action.

The first group consists of agents known collectively as non-selective liberators and is best illustrated by detergents such as Triton X-100 and Tween-20 [53]. They are

cytotoxic and act by disrupting the mast cell plasma membrane, thereby causing irreversible damage to the cell and facilitating the release of all the intracellular contents including histamine.

In contrast, agents belonging to the second group are non-cytotoxic and can cause release without the loss of characteristic cytoplasmic markers such as lactate dehydrogenase. These selective liberators include polybasic cations typified by compound 48/80 [54], peptide 401 [55], polylysine [56] and polymyxin [57]. The group also contains agents such as the anaphylatoxins C3a, C4a and C5a [58,59], calcium ionophores [60,61], the plasma substitute dextran [62], adenosine-5'-trisphosphate (ATP) [63] and a diversity of drugs and organic compounds [2]. These selective liberators, especially the polybasic compounds, have proved extremely useful in studying the biochemical events involved in cell activation [64] and in the functional studies of mast cell heterogeneity [65,66].

1.3.3 Ultrastructural changes during mast cell activation

The ultimate aim of mast cell activation is the release of chemical mediators into the external milieu. This is generally achieved by the fusion of the membrane surrounding the granule with the membrane surrounding the whole cell. Such a process is an example of a phenomenon known as exocytosis.

The sequence of events leading to exocytosis may be dissimilar for different mast cell types. In the rat peritoneal mast cell, the initial ultrastructural change involves those granules situated immediately below the cell membrane. These granules become swollen in appearance and the release process begins via openings produced by the fusion of the two membranes. Following this initial contact, many intergranular fusions are formed which spread towards the interior of the cell and open up extensive labyrinthic cavities within the cell which can communicate with the extracellular medium through multiple openings. As granules are discharged, histamine and other ionically-bound molecules are then released, possibly through a simple exchange process with external sodium ions [67,68].

The mechanism of human mast cell degranulation has been shown to differ somewhat from its rat counterpart. In human lung mast cells, the fusions between individual granules result in the formation of many degranulation channels. As these channels expand, granule contents begin to leave the cell via multiple openings produced by the fusions of degranulation channels with the cell membrane. However, unlike rat peritoneal mast cells, no apparent extracellular expulsion of granules is observed [24,69,70]. Recovery of the mast cells can be divided into two stages, with the whole process taking up to 48 hr. The early recovery phase involves resealing of degranulation openings and the shrinking of degranulation channels [71]. The late phase involves blast formation characterized by nuclear enlargement and the appearance of nucleoli. This is then closely followed by the synthesis of new granules [72].

1.4 Mast cell mediators

Mast cells activated by both immunological and non-immunological stimuli may release a wide range of inflammatory mediators which give rise to the symptoms of anaphylaxis. These mediators may either be preformed and stored within the secretory granules or be synthesized de novo upon activation.

1.4.1 Preformed mediators

1.4.1.1 The biogenic amines

Histamine (from the Greek word for tissue, histos) is synthesized in nature from histidine by the enzyme histidine decarboxylase [73] and has now been recognised to be the dominant biogenic amine stored in mast cells. Rat serosal mast cells contain 10-30 pg per cell [74,75], rat mucosal mast cells 1-2 pg [65], human mast cells [66,76-78] and human basophils 1-3 pg [79].

Once released into the extracellular medium, histamine exerts its biological effects by binding to three sub-classes of histamine receptor, the H_1, H_2 and H_3 sub-types [80,81]. The H_3-receptor has been most recently described in the central nervous system [81] but its involvement in histamine-mediated effects in the periphery has yet to be

clarified. Amongst the effects mediated via the H_1-receptor are contraction of bronchial and gastrointestinal smooth muscle, vasodilatation, increased capillary permeability and neutrophil and eosinophil chemokinesis [82]. In contrast, the effects mediated via the H_2-receptor are more anti-inflammatory and include the inhibition of T-lymphocyte cytotoxicity, suppression of lymphocyte proliferation, inhibition of lysosomal enzyme release from neutrophils and stimulation of gastric acid secretion [83]. Moreover, many other biological effects, although predominantly H_1 or H_2-mediated, result from the combined effects of histamine binding to both receptor sub-types.

Despite its wide range of activities, histamine is unstable, having a plasma half-life of 2-3 min. Metabolism occurs via two routes, N-methylation by histamine-N-methyltransferase or oxidation by histaminase (diamine oxidase).

Serotonin, or 5-hydroxytryptamine is stored with histamine in granules of murine mast cells [74,75,84] while human mast cells have been shown to be deficient in this amine [85,86]. Serotonin is a well-recognised neuro-transmitter in the central nervous system. Peripherally, it enhances vasopermeability in rodents but not in humans and can elicit vasoconstriction and vasodilatation in distinct vascular beds [87,88].

1.4.1.2 Proteoglycans

The characteristic metachromatic staining observed when mast cells are treated with basic dyes depends on the presence of highly sulphated proteoglycans in the secretory granules [10,89,90]. Proteoglycans are a major component of tissue ground substance and comprise a central protein core from which long carbohydrate side chains are attached. Two major sub-classes have been identified in mast cells; these are heparin and the chondroitin sulphates.

Heparin consists of a central peptide core, comprising alternating serine and glycine residues. Glycosaminoglycans (GAGs) are attached to every second or third serine residue by a unique sequence of sugars, comprising glucuronic acid-galactose-galactose-xylose, a linkage that is common to all proteoglycans [91]. The GAG is made up of a series of disaccharide units that are $\alpha_{1\text{-}4}$ linked and composed of either

glucuronic or iduronic acid in β_{1-4} linkage to glucosamine (fig 1.2) [92,93].

Mast cells are the sole source of extracellular heparin, and this molecule has the unique property of inactivating other stored preformed mediators and to package them in an orderly array. Histamine is bound to the carboxyl groups of proteins ionically linked to heparin [94], whereas the neutral proteases and other cationic mediators bind to the anionic sulphate group of the GAGs [95,96]. Apart from the above property, heparin is also a potent anticoagulant [97], has anticomplementary activity [98] and can act as an inhibitor of the enzymes plasmin and kallikrein [99].

Apart from heparin, the chondroitin sulphates are a group of similarly related proteoglycans which are also located in mast cells [100-102]. Chondroitin sulphates are glycosaminoglycans with β_{1-4} linked disaccharide units of glucuronic acid in β_{1-3} linkage to galactosamine. However, the distribution of the chondroitin sulphates depends principally on the locality of the mast cells. Indeed, the same also applies to heparin and this theme will be discussed further in the section on mast cell heterogeneity.

1.4.1.3 Other preformed mediators

Apart from the biogenic amines and the proteoglycans, mast cell granules also contain an extensive variety of enzymes. These may be hydrolytic in nature such as β-hexosaminidase, β-glucuronidase and arylsulphatase. They are loosely bound to the structural matrices and are released in parallel with histamine during cell activation. Moreover, in phagocytic leukocytes, these enzymes serve an important role in the lysosomal degradation of ingested materials, and there is some evidence that a similar mechanism may be preserved in mast cells [103].

Mast cell granules also contain a group of proteolytic enzymes termed collectively as neutral proteases (enzymes that cleave peptide bonds with maximal activity at neutral pH). In both rat and human mast cells, they comprise up to one third of the total protein of the cell and therefore assume some importance in the immediate hypersensitivity response. In addition, the recognition that both rat and human mast cells isolated from different anatomic sites exhibit a different spectrum of neutral

protease activity has provided convincing evidence for the existence of mast cell heterogeneity [104]. The majority of neutral protease activity of rodent mast cells resides among enzymes exhibiting chymotryptic specificity, whereas in human mast cells enzymes of both tryptic and chymotryptic natures have been confirmed [89,105-107].

Research into the roles of the neutral proteases is still in its infancy. However, apart from their proteolytic activities, human tryptase has been shown to be able to generate the anaphylatoxin C3a from C3 [108], an important component of the complement cascade, while both rat and human chymase can convert angiotensin I to angiotensin II with high activity [109].

Mast cells can also release substances which are chemotactic for eosinophils and neutrophils. The eosinophil chemotactic factor of anaphylaxis (ECF-A) comprises a family of oligopeptides of relative molecular mass 300 - 5,000 [75,110]. Moreover, release of these factors has been observed following immunological activation of human [111] and guinea pig [112] lung tissues and from rat peritoneal mast cells [113]. In contrast, neutrophil chemotactic factor of anaphylaxis (NCF-A) is a much larger protein of relative molecular mass 600,000 - 750,000 [114,115]. Release of both ECF-A and NCF-A will induce infiltration of the secondary inflammatory cells which is a characteristic of the late phase reaction of many allergic disorders.

1.4.2 Newly generated mediators

In addition to the preformed mediators, mast cells can also synthesize and release a variety of bioactive agents known collectively as the eicosanoids during both immunological and non-immunological activation. This process begins when arachidonic acid (5,8,11,14-eicosatetraenoic acid) is released from the membrane phospholipids by the action of the enzyme phospholipase A_2. Once liberated, arachidonic acid is metabolized to produce either prostaglandins (PGs) and thromboxanes (TXs) through the cyclooxygenase pathway or leukotrienes (LTs) via the lipoxygenase pathway (fig 1.3) [75,110].

Cyclooxygenase is a membrane associated heme protein enzyme complex that catalyzes the incorporation of molecular oxygen into the arachidonic acid molecule, followed by ring closure to form the relatively unstable cyclic endoperoxide intermediates PGG_2 and PGH_2. These intermediates may then be converted to yield the primary prostaglandins PGD_2, PGE_2 and $PGF_{2\alpha}$, in addition to PGI_2 (prostacyclin) and TXA_2 [116,117]. In both human and rat mast cells, the major cyclooxygenase product released following activation is PGD_2, while the others are produced in much smaller quantities [77,118-121].

Leukotrienes are generated in another pathway by the action of the enzyme 5-lipoxygenase. The initial product is the unstable intermediate 5-hydroperoxyeicosatetraenoic acid (5-HPETE) which in turn is quickly metabolized to 5-hydroxyeicosatetraenoic acid (5-HETE) and LTA_4. The latter is rapidly converted to LTB_4 by reaction with water, or with the tripeptide glutathione, to form the sulphidopeptide leukotrienes LTC_4, LTD_4 and LTE_4 which comprise the biological activity of the slow reacting substance of anaphylaxis (SRS-A) [122-124].

The biological actions of the eicosanoids are extensive. PGD_2, $PGF_{2\alpha}$, LTC_4, LTD_4 and TXA_2 represent the most powerful contractile agents tested in human airways both in vitro and in vivo [125] while in contrast, PGE_2 relaxes airway smooth muscle [125]. PGE_2 and PGI_2 both augment vascular permeability and along with PGD_2 are powerful vasodilators [125]. PGD_2 and $PGF_{2\alpha}$ are chemokinetic for neutrophils and eosinophils and are potent inhibitors of platelet aggregation [75,125]. TXA_2 in contrast, is a powerful platelet aggregator [125].

On the theme of platelet aggregation, there is evidence that mast cells, especially those cultured and differentiated in vitro from mouse bone marrow in the presence of interleukin 3, can synthesize a unique phospholipid named platelet activating factor (PAF) (1-0-alkyl-2-acetyl-sn-glyceryl-phosphorylcholine) [126]. In addition, a recent study by Schleimer et al [127] has suggested that highly purified human lung mast cells can generate this mediator upon immunological activation. Although PAF was originally characterized by its potent aggregatory effects on platelets [125,128], it has now been demonstrated to be involved in a number of pathological conditions. The evidence

pointing towards a possible role in asthma is especially convincing, for in addition to inducing some of the symptoms of asthma in animals and patients, PAF is also generated in the course of an asthmatic attack, presumably mainly from eosinophils which appear especially equipped to produce this material.

1.5 Biochemical events involved in mediator secretion

Mast cell activation and the subsequent release of chemical mediators involves a number of inter-related biochemical events. However, it is important to bear in mind that most of the current information has been obtained from studies with murine mast cells and that these data do not always necessarily extend to human cells.

1.5.1 The role of calcium

1.5.1.1 Calcium and histamine secretion

The intracellular concentration of free calcium ions in most cell types is around 0.1 μM, whereas the concentration of the cation in the extracellular medium is considerably higher (1 mM). This large concentration gradient is maintained by the relative impermeability of the plasma membrane to calcium and by the efficient transport of the cation out of the cell [131].

Recognition of the role of the calcium ion as a ubiquitous second messenger in linking mast cell activation to the subsequent mediator release was probably first demonstrated by the work of Mongar and Schild [132], who showed that optimal anaphylactic secretion of histamine in fragments of guinea pig lung required the presence of this divalent cation in the bathing solution.

More recently, evidence for the involvement of calcium in mediator secretion was further provided by Kanno and his co-workers [133]. They showed that histamine release could be induced by direct microinjection of the cation into rat mesenteric mast cells. This effect was specific for calcium and could not be reproduced by potassium or magnesium ions nor by direct mechanical insult. Histamine release can also be

induced by fusing mast cells with liposomes containing calcium [134] or by treatment with calcium ionophores [135,136]. The latter are organic molecules which assist the transfer of cations across biological membranes. They comprise a hydrophilic interior capable of complexing with given metal ions. The lipophilic exterior of the ionophore facilitates partition of the complex into the cell membrane and promotes transport of the ion across the membrane along its concentration gradient.

Lanthanum ions and, to a lesser extent, other members of the lanthanide series have also been used to demonstrate this calcium dependency [137,138]. Lanthanum has an ionic radius comparable to that of calcium and is able to displace competitively the divalent cation from superficial sites in the cell membrane. By virtue of its higher valency, lanthanum binds to these sites with a greater affinity than calcium and blocks subsequent movements of calcium across the membrane. Employed in this way, it has been shown that lanthanum inhibits histamine release induced by various secretagogues [138].

Moreover, studies using radioisotopic calcium in both rat peritoneal [139] and human lung mast cells [140] have shown a net increase in cytosolic calcium following immunological activation. More recently, this work has been extended by the use of the fluorescent tetracarboxylate calcium chelator quin-2 [141].

1.5.1.2 Calcium pools involved in histamine secretion

It is now well established that a rise in the concentration of free calcium ions in the mast cell cytosol is a necessary and sufficient trigger for histamine secretion. For many agents employed in mast cell research, optimal release of histamine requires the presence of this divalent cation in the extracellular medium [136]. The general theory suggests that the process begins by the binding of the cation to superficial sites on the cell membrane. Subsequently, this pool of calcium rapidly equilibrates with the extracellular environment and eventually migrates into the cell through appropriate channels to initiate secretion. Lanthanide ions, as described above, presumably exert their action by binding to and blocking these sites [136].

Evidence for this hypothesis has been derived from the finding that rat mast cells isolated in the presence of calcium and then diluted by addition to a medium free of the divalent cation, display a progressive decrease in the level of histamine release as the time interval between dilution and challenge with test secretagogues is increased [131,142]. In addition, these results were corroborated by the use of kinetically slower secretagogues, such as the IgE-directed ligands and suboptimal concentrations of A23187, to allow dissociation of the ion from the membrane, upon dilution, to proceed more rapidly than the ability of the secretagogue to mobilize this calcium pool. Consistently, an immediate reduction in release was observed upon transfer of the cells into calcium-free solutions containing these secretagogues, followed by a further progressive decay [131].

Although optimal histamine secretion requires the presence of extracellular calcium ions, many test secretagogues can release a considerable amount of this amine in the absence of the cation [143]. The best examples to illustrate this effect are the polybasic compounds such as compound 48/80, polylysine and peptide 401 [135,136,144]. The resultant release is attributed to the mobilization of intracellular or sequestered calcium which might be deeply buried within the cell membrane, attached to the inner surface of the membrane or located within the endoplasmic reticulum or mitochondria. Moreover, removal of intracellular calcium by preincubating cells with chelating agents such as ethylenediaminetetraacetic acid (EDTA) in calcium-free buffer renders them unresponsive to subsequent stimulation by secretagogues [135,136,145]. These cells, however, regain partial or full responsivity when transferred back to a medium containing calcium.

Although prolonged treatment with EDTA renders the mast cells unreactive to subsequent challenge by secretagogues, brief (5 min) exposure to this chelating agent markedly enhances the secretion produced by many inducers [136]. This treatment is believed to remove calcium ions from superficial, regulatory sites in the cell membrane, leading to its destabilization and thus facilitating the release of more internal stores of the cation. In total contrast, high concentrations of extracellular calcium have the opposite effect, the result is saturation of these regulatory sites, thus stabilizing the cell membrane and possibly restricting uptake of the cation [136].

1.5.1.3 Calcium and calmodulin

Although an increased concentration of free calcium in the cell cytosol is a necessary and sufficient trigger for mediator secretion from the mast cell, the actual mechanism behind this process is still not fully understood. However, results from other cell systems have shown that many of the effects of calcium are mediated through a calcium regulatory protein, termed calmodulin (CaM) [146-148].

CaM is ubiquitously distributed in eukaryotic cells, and its structure has been found to be nearly identical in many species ranging from simple coelenterates to man [136,146,149]. It has a relative molecular mass of about 17,000 and is highly acidic. Each molecule of CaM can bind up to four calcium ions in a reversible fashion and with high affinity. Upon binding, the protein undergoes significant conformational changes, resulting in an increase in its helical content and a more stable structure. These changes expose certain hydrophobic sites on its surface through which it can combine with and activate target apoenzymes or functional proteins.

In this way, CaM mediates the control of a large number of important enzymes, including cyclic nucleotide phosphodiesterase, adenylate and guanylate cyclases, methyltransferase, phospholipase A_2, the ATPase and calcium pump of the plasma membrane and sarcoplasmic reticulum, myosin light-chain kinase and a variety of other kinases [136,147,148]. With this in mind, CaM therefore controls many key events involved in exocytosis, including the regulation of calcium homeostasis, the synthesis and degradation of cyclic nucleotides, the organization of the cell cytoskeleton and protein phosphorylation [136,147-149].

The role of CaM in protein phosphorylation merits further comments. It has been demonstrated that pharmacological activation of rat peritoneal mast cells results in a rapid, calcium-dependent phosphorylation of three proteins of relative molecular masses 42,000, 59,000 and 68,000 [19,150]. This phosphorylation occurs before histamine secretion from the cell. The termination of the release process is connected with the phosphorylation of a further protein of relative molecular mass 78,000. Therefore, calmodulin, by its control over a number of kinases, may be involved in both the

induction and termination of histamine secretion, although the validation of this hypothesis remains to be demonstrated.

The involvement of CaM in exocytosis is further confirmed by the finding that CaM inhibitors, which include the naphthalene sulphonamide (W7) and various neuroleptic drugs such as the phenothiazines, inhibit histamine release from both mast cells and basophils [151-153].

1.5.2 Phospholipid metabolism

In recent years, much attention has been focused on the phospholipids of the mast cell membrane and how changes in these lipids might play a part in the release process.

1.5.2.1 Phosphoinositide breakdown

It has been demonstrated from a large number of cell systems that many agonists which use calcium as a second messenger also evoke parallel changes in the metabolism of the endogenous membrane lipid phosphatidylinositol (PI) [154]. In early studies, PI breakdown was measured experimentally by analysing the incorporation of radiolabelled precursors such as [^{32}P] phosphate and [^{3}H] inositol into PI, phosphatidylcholine and phosphatidic acid. Indeed, in rat mast cells, several workers [155-157] have demonstrated that a number of secretagogues including IgE-directed ligands, compound 48/80, calcium ionophore A23187 and chymotrypsin cause an accumulation of [^{32}P] phosphate into mast cell PI. More recently, Beaven et al [158] have shown IgE-mediated PI turnover in RBL cells while Ishizaka et al observed the same phenomenon with cultured basophils [159].

Interest in this field has now shifted to the potential role of polyphosphoinositide (PPI) breakdown and its effect on calcium mobilization [160,161]. Phosphorylation by ATP-dependent specific kinases converts PI first to the 4-monophosphate (PIP) followed by the 4,5-bisphosphate (PIP_2) (fig 1.4). Receptor activation in the membrane activates a phospholipase C probably through a GTP-binding regulatory protein. The phospholipase C converts PIP_2 into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol

(DAG). IP_3 is rapidly transformed to either inositol 1,4-bisphosphate (IP_2) or, alternatively, to inositol 1,3,4,5-tetrakisphosphate (IP_4) (fig 1.4).

The products of PIP_2 breakdown, namely IP_3 and DAG, are important second messengers in the signal transduction process. The former product has been shown to release calcium from a non-mitochondrial pool which has characteristics that suggest that it is likely to be the endoplasmic reticulum [160-162]. Moreover, various studies using permeabilized cells have shown that submicromolar concentrations of IP_3 are required for half-maximal calcium release from these stores [163]. Recently, IP_3 together with IP_4 have been implicated in the regulation of calcium entry [161].

The above theme also leads to the controversial topic of the calcium-dependence of the phosphoinositide turnover. Early studies have indicated that PI turnover in mast cells stimulated by Fc_ε receptor cross-linking was independent of extracellular calcium [156]. This is an important point since if an inositol phosphate such as IP_3 is responsible for the generation of the calcium signal, the formation of the inositol phosphate should not itself be calcium-dependent. However, results obtained from RBL cells showed that the hydrolysis of inositol phospholipids following cell activation was dependent on the presence of extracellular calcium [158] and was stimulated by calcium ionophores [164]. From this, the exact relationship between phosphoinositide turnover and the subsequent generation of the calcium signal is not entirely clarified.

The other product of PIP_2 breakdown, DAG, has widely been accepted as an activator of protein kinase C (PKC) [165,166], a calcium and phospholipid-dependent enzyme which has been detected in a variety of tissues [167] and also in the cytoplasm of mast cells [168]. DAG activates PKC which catalyzes phosphorylation of serine and threonine residues in proteins, in the presence of phospholipids, by reversibly increasing its affinity for calcium ions such that it can be activated at submicromolar concentrations of this cation [169]. DAG, in this way, could thus serve as a second messenger to activate protein phosphorylation.

Various observations have suggested a potential role for PKC in mast cell stimulus-secretion coupling. Firstly, IgE-dependent stimulation of rat mast cells [170],

mouse cultured mast cells [171] and human cultured basophils [159] caused a rapid accumulation of DAG which preceded or was concurrent with histamine release. Secondly, White et al [171] have shown that antigen-activation of mouse cultured mast cells stimulated PKC activity associated with the cell particulate fraction. Kinase activation was maximal 30 s after challenge and preceded histamine secretion, and both enzyme activation and histamine release were similarly dependent on antigen concentration.

Further evidence was provided by the use of phorbol esters such as phorbol myristate acetate (PMA) and tetradecanoyl phorbol acetate (TPA), agents which mimicked the effects of DAG on PKC. At low concentrations (<10 ng/ml), these compounds were found to be poor histamine releasers, but potentiated histamine secretion activated by sub-optimal concentrations of IgE-directed ligands and calcium ionophores in human basophils [172,173], rat peritoneal mast cells [168,174] and RBL cells [175]. These results thus suggest that PKC has a positive modulatory role in histamine secretion. Therefore, the two products of PIP_2 breakdown (IP_3 and DAG) may represent two routes of a second messenger pathway which positively interact with each other.

However, that PKC can also negatively modulate secretion was suggested by observations with RBL cells. Sagi-Eisenberg et al [176] have shown that at low concentrations (<10 ng/ml), TPA potentiated antigen-induced serotonin release but, in contrast, completely blocked the intracellular calcium increase normally associated with this secretagogue. The concept of PKC acting as a negative modulator of signal transduction is further supported by observations that, in the mast cell [177] and in a variety of other cell types [178,179], phorbol esters can inhibit phosphoinositide breakdown and calcium mobilization. These observations may be interpreted either in terms of PKC having two roles: one to interact positively with the calcium signal and one to suppress it or that phorbol esters may exert an effect other than that through PKC activation.

1.5.2.2 Phospholipid methylation

An alternative pathway of membrane activation involves the methylation of endogenous phospholipids [180,181]. According to this scheme (fig 1.5), two membrane-bound methyltransferases (I and II) sequentially convert phosphatidylethanolamine (PE) to phosphatidylcholine (PC) by successive N-methylation. Methyltransferase I is located on the cytoplasmic face and transfers a methyl group from the donor S-adenosyl-L-methionine to PE to form phosphatidyl-N-monomethylethanolamine. The enzyme is dependent on magnesium ions. Methyltransferase II is located on the external surface of the bilayer and catalyzes two successive N-methylations of phosphatidyl-N-monomethylethanolamine to form PC. PC may then be a substrate for phospholipase A_2 which converts it to lyso PC and arachidonic acid. The formation and transfer of methylated phospholipids increases the fluidity of the cell membrane and this change in microviscosity may be associated with an enhanced permeability to calcium ions.

The methylation of phospholipids has been demonstrated following IgE-mediated activation of human pulmonary mast cells [140,182], murine peritoneal mast cells [183,184] and RBL cells [185]. Indeed, this phenomenon appears to occur before several other biochemical changes associated with IgE-dependent stimulation such as cAMP accumulation, ^{45}Ca uptake and, ultimately, histamine secretion. Moreover, inhibitors of methyltransferases such as 3-deazaadenosine (3-DZA) and homocysteine thiolactone (HC) blocked phospholipid methylation, calcium influx and histamine release in a parallel, highly correlated fashion [182,185,186].

Further evidence in favour of a role for phospholipid methylation in IgE-mediated release has come from studies with variants of the RBL cell line [187]. Two drug-resistant sublines were produced that were unable to take up calcium or to release histamine on immunological stimulation. However, these cells released the amine on challenge with calcium ionophores indicating that their secretory mechanism distal to the activation of calcium channels was intact. Detailed study showed that one cell line was deficient in the enzyme methyltransferase I and the other in methyltransferase II. Fusion of the mutants produced hybrids with normal phospholipid methyltransferase activity and restored the ability of the cells to respond to IgE-mediated challenge with calcium uptake and histamine release.

It should be noted, however, that the increase in phospholipid methylation appears to be confined essentially to IgE-directed ligands. Indeed, stimulation of rat mast cells with the ionophore A23187 or compound 48/80 produced no appreciable changes [183]. Similarly, inhibitors of methyltransferases did not prevent histamine release from rat mast cells treated with these agents, polymyxin B or ATP.

The mechanism whereby phospholipid methylation leads to calcium influx and secretion remains the source of speculation. The increase in membrane fluidity may directly increase the permeability to calcium ions or facilitate the opening of calcium channels [189] or may activate an ecto-ATPase, thought by some to be involved in the transport of calcium into the mast cell [190]. Alternatively, the generation of lyso-PC, a known fusogen [191], may facilitate the membrane changes involved in exocytosis or the opening of calcium channels.

Finally, more recent findings have raised serious doubts concerning the relevance of phospholipid methylation to mast cell secretion. Firstly, a number of groups have failed to confirm the reported increase in phospholipid methylation following activation of mast cells or leukaemic basophils [19,192-194] and secondly, methyltransferase inhibitors such as DZA may have other effects distal to the methylation process [194].

1.5.3 Arachidonic acid metabolism

The activation of phospholipase A_2 (PLA_2), or phospholipase C (PLC) in concert with diacylglycerol lipase, generates free arachidonic acid which may be metabolised by the cyclooxygenase and lipoxygenase pathways to form prostaglandins, leukotrienes and thromboxanes (section 1.4.2). It has been proposed that these metabolites, apart from their inflammatory effects, may also be involved in the modulation of the release process.

It has been demonstrated that highly purified PLA_2 preparations caused non-cytotoxic release of histamine from mast cells [195] and human basophils [196]. Moreover, the release was blocked by inhibitors of PLA_2, namely p-bromophenacylbromide (BPB), mepacrine and tetracaine [195,197,198].

The acetylinic analogue eicosa-5,8,11,14-tetraynoic acid (ETYA), which blocked both the cyclooxygenase and lipoxygenase pathways, caused a dose-dependent inhibition of histamine release from rat mast cells, human lung mast cells and human basophils [197,199-201]. More specific inhibitors of the lipoxygenase pathway such as 5,8,11,14-henicosatetraynoic acid (ITYA) and 5,8,11-eicosatriynoic acid (ETI) also inhibited secretion [200,202]. In total contrast, non-steroidal anti-inflammatory drugs such as aspirin, indomethacin and meclofenamic acid, which blocked only the cyclooxygenase pathway, had no effect or even potentiated release [197,200,203]. From this, it was suggested that one or more products of the lipoxygenase pathway are involved in the modulation of histamine secretion. Possible candidates for this role are 5-hydroxyeicosatetraenoic acid (5-HETE) and 5-hydroperoxyeicosatetraenoic acid (5-HPETE) since they produced dose-dependent enhancement of IgE-mediated histamine release [204,205]. 5-HETE has also been suggested to act as an ionophore [206] and to enhance or to prolong the activation of protein kinase C.

However, more recent observations have raised doubts over the concept that activation of the lipoxygenase pathway may be required for the release of histamine from mast cells and basophils. It has been repeatedly demonstrated that certain stimuli [208,209] failed to initiate the synthesis of LTC_4 despite the release of substantial quantities of histamine, while L651-392 (4-bromo-2,7-dimethoxy-3-H-phenothiazine-3-one), a specific inhibitor of the lipoxygenase pathway, failed to inhibit histamine release from both purified human basophils and human lung mast cells [210].

1.5.4 The role of cAMP

It is now firmly established that the cyclic nucleotides adenosine 3',5'-cyclic monophosphate (cAMP) and guanosine 3',5'-cyclic monophosphate (cGMP) are involved in the modulation of biological responses in a number of cell types. In particular, cAMP may act as a second messenger in the regulation of calcium homeostasis [136,211,212]. In an attempt to rationalize the diverse effect of cAMP, Berridge [211] has divided the possible control mechanisms into two main categories: monodirectional and bidirectional. In monodirectional systems, cAMP acts to enhance the secretion induced by calcium. The increased intracellular level of the nucleotide is typically

generated by a direct effect of the secretagogue on the adenylate cyclase and activation of the cell is accompanied by an increase uptake of calcium and a rise in the concentration of cAMP [211]. In bidirectional systems, cAMP is believed to inhibit the calcium-induced response by activating membrane pumps (calcium ATPases) which promote extrusion of the cation from the cytosol either into the external environment or into intracellular stores.

It has long been appreciated that agonists which alter mast cell and basophil cAMP levels regulate mediator release. From early studies in guinea pig lungs, high doses of adrenaline were demonstrated to inhibit antigen-induced histamine release [213]. Similarly, in human lung fragments, histamine release was inhibited by agents which elevated cAMP [214] and the same phenomenon was observed in isolated human mast cells [76,215,216] and human basophils [217,218]. These findings would suggest that the IgE-dependent increase in cAMP is inhibitory to secretion, possibly constituting a negative feedback mechanism. In contrast, in rat serosal mast cells, no consistent relationship was obtained between changes in cAMP levels and inhibition of mediator release. For example, β-adrenoceptor agonists caused little or no inhibition of IgE-dependent histamine secretion despite raising cAMP levels [219,220]. However, inhibitors of cAMP phosphodiesterase, such as theophylline or 3-isobutyl-1-methylxanthine (IBMX), which prevent the breakdown of endogenous cAMP, decreased mediator secretion by both IgE-dependent and non-immunological stimuli [221-223]. The studies of Holgate et al [224] further demonstrated the complex relationship between cAMP and mediator secretion. These workers found that neither PGD_2 nor PGI_2 alone inhibited IgE-dependent mediator secretion despite raising cAMP 2-3 fold, whereas theophylline alone produced a concentration-dependent inhibition of mediator release that was inversely correlated to mast cell cAMP levels. Although theophylline enhanced PGD_2-dependent increases in cAMP, IgE-dependent histamine release was inhibited no more than with theophylline alone. From these results, a hypothesis was put forward to suggest that several distinct pools of adenylate cyclase may exist in rat mast cells which are differentially affected by stimuli and drugs, and that only certain pools are linked to the modulation of mediator release.

Studies of the changes in cAMP levels following activation of the mast cell have

provided further evidence in favour of the above model and have also yielded important information concerning the mechanism of the release process. Cross-linkage of the IgE-receptors on rat serosal mast cells [193,225,226], human lung mast cells [140] and human basophils [149,227,228] was associated with increases in cellular cAMP levels. In each case, the early increase in the cyclic nucleotide was transient, reaching a maximum at 15-45 s following challenge. A later rise in cAMP in rat mast cells could also be observed within 3-5 min of cell activation. This later increase was suppressed by indomethacin, and was probably due to adenylate cyclase stimulation by the release of newly synthesized PGD_2 [225]. From this, the early change in cAMP was considered to be involved in stimulus-secretion coupling.

It then remains to be decided whether the rise in cAMP following receptor bridging is involved in the triggering or termination of the release process. In eukaryotic cells, the effects of the cyclic nucleotide are mediated through the activation of protein kinases [229]. In rat mast cells, two such enzymes (designated Type I and Type II) have been identified [230]. IgE-dependent activation of rat serosal mast cells produced a significant increase in protein kinase activity at 60 s after challenge [230], with both enzymes being activated to a similar degree. Theophylline, which increased cAMP and inhibited IgE-mediated secretion, activated protein kinase whereas PGD_2 raised cAMP but had little effect on secretion or kinase activation [231]. When added together, these agents increased kinase activity and inhibited mediator release no more than theophylline alone, demonstrating a correlation between kinase activation and inhibition of mediator release.

In conflict to the above findings, other observations suggest that increases in cAMP might facilitate secretion. Holgate et al [232] have shown that the ability of ribose and purine modified analogues of adenosine to inhibit or enhance IgE-dependent increases in cAMP correlated with their ability to inhibit or enhance mediator secretion. Moreover, in human basophils [233] and rat mast cells [231], 2',5'-dideoxyadenosine (DDA) inhibited IgE-dependent increases in cAMP and mediator secretion.

It has also been suggested that the time-course of changes in cAMP relative to challenge might decide the final effect on mediator secretion. In support of this

hypothesis, in rat mast cells, the β-adrenoceptor agonist salbutamol or the adenylate cyclase activator forskolin inhibited antigen-dependent secretion when added 2-5 min prior to challenge, enhanced secretion when added simultaneously or had no effect when added after challenge [163].

In view of all these contradictory observations, and in the light of more recent observations, it is possible that IgE-dependent increases in cAMP have no important role in the release process. Firstly, in RBL-2H3 cells, which possess a functional adenylate cyclase, IgE-dependent activation does not increase cAMP, although increases in phosphoinositide breakdown and calcium influx are evident [158,234]. Secondly, in human lung mast cells, Peachell et al [235] have been unable to demonstrate IgE-dependent increases in cAMP, in contrast to the previous finding of Ishizaka et al [140]. Thirdly, non-immunological stimuli which are effective secretagogues in rat mast cells do not increase cellular cAMP [236,237]. This latter observation would suggest that, if cAMP has any role in IgE-dependent secretion, then this occurs at an early stage in stimulus-secretion coupling which is not shared by these other stimuli.

1.5.5 Phosphorylation of cellular proteins

As mentioned previously, stimulation of the mast cell results in the activation of a number of protein kinases which in turn causes the phosphorylation of cellular proteins. The activation of rat mast cells with anti-IgE, compound 48/80 or the calcium ionophore A23187 results in the rapid phosphorylation, via a calcium-dependent reaction, of proteins with relative molecular masses of 42,000, 59,000, 68,000 and 78,000 [238-241]. The phosphorylation of the 42,000, 59,000 and 68,000 proteins can be observed at a time when histamine release is starting; however, phosphorylation of the 78,000 protein is much slower and occurs after cell secretion. The proteins are then dephosphorylated at different rates. The addition of cromolyn and several other inhibitors of histamine release transiently increases the phosphorylation of a 78,000 protein which might be identical to that induced by cell activation [239,240]. These results have led to the proposal that phosphorylation of the former three proteins is involved in the onset and or continuation of secretion, whilst that of the 78,000 protein is involved in the termination of secretion.

1.5.6 Activation of serine esterases

It has been suggested that activation of one or more proteolytic enzymes might constitute the earliest biochemical change that ultimately leads to mediator secretion from the mast cell. Austen and co-workers have demonstrated that diisopropyl fluorophosphate (DFP), an irreversible inhibitor of serine esterases, blocked IgE-dependent histamine release from rat serosal mast cells [242,243] and from chopped guinea pig and human lung fragments [244,245]. They have also shown that histamine release was inhibited only when DFP was present at the time of challenge, its effect being greatly reduced if the cells were incubated with the inhibitor and washed prior to the addition of the stimulus. This implies that the enzyme is activated only after the start of the secretory process. Detailed studies on purified rat peritoneal mast cells [246] revealed that DFP blocked not only histamine release but also prevented other biochemical events such as calcium uptake, phospholipid methylation and the transient rise in cAMP.

In further support of a potential role for serine esterases, α-chymotrypsin [247] and rat mast cell chymase [248] induced mediator secretion from rat mast cells. Furthermore, substrates and inhibitors of trypsin and chymotrypsin have been shown to inhibit IgE-dependent phospholipid methylation and the cAMP rise [234].

1.6 Mast cell heterogeneity

Due to their abundance and simplicity of purification, rat peritoneal mast cells were employed in most early pharmacological and biochemical studies. However, with the development of methods for the enzymic dispersion and isolation of free mast cells from a number of target tissues including the heart [249], intestine [65,76-78], lung [76,216,250], mesentery [251] and skin [252,253], a rapidly increasing body of evidence now firmly indicates that mast cells from different species and even from different tissues within a given animal may exhibit marked variations in their biochemical, histochemical and functional properties.

1.6.1 Histochemical differences between mast cells

Maximow [254] was probably the first person to recognise that certain mast cells in the rat intestinal mucosa were atypical in their staining characteristics and differed from those observed in other anatomical sites. This work was greatly extended by Enerbäck and his co-workers who identified two distinct types of mast cells in the gastrointestinal tract of the rat [255,256]. The mast cells in the lower layer of the intestinal wall resemble those in other connective tissues and the serosal cavities (connective tissue mast cell(s); CTMC), whereas the cells in the mucosa (mucosal mast cell(s); MMC) display different properties (table 1.1). They are smaller in size, more variable in shape, have a lower content of histamine and serotonin. MMC also possess fewer granules than CTMC and these contain the less highly sulphated glycosaminoglycan chondroitin sulphate rather than heparin [257].

A characteristic feature of the mast cell is its ability to stain metachromatically with certain cationic dyes such as toluidine blue. This metachromasia arises from a shift in the colour spectrum of the dye following the interaction with the anionic proteoglycan granular matrix. In this respect, due to the difference in their proteoglycan structure, MMC differ from CTMC in that special conditions of fixation and staining are required to demonstrate their presence. Indeed, the granules of MMC unlike those belonging to the CTMC may become resistant to metachromatic staining after preservation in some common formalin-based fixatives [256].

Differences in the charge distribution of the proteoglycan matrix may also be revealed by staining with combinations of dyes such as alcian blue and safranin. The mature rat CTMC stain metachromatically with safranin whereas the MMC of the same animal stain orthochromatically with alcian blue, consistent with the lower degree of sulphation of their proteoglycan matrix [257]. In a similar fashion, berberine sulphate, a fluorescent dye, forms a strongly fluorescent complex with heparin in CTMC but not MMC granules [258].

It must be noted that the above histochemical criteria for distinguishing between subpopulations of mast cells have been developed exclusively for the rat. In man, however, the situation is less clear. A detailed study by Strobel et al [259] has demonstrated two subtypes of mast cell in the intestine based on their formalin

sensitivity. A further study using formalin and Mota's lead acetate fixative demonstrated that the small intestinal lamina propria contained predominantly formalin-sensitive mast cells whereas the submucosa and muscle contained mostly formalin-resistant cells [260]. In the large intestine, these two subtypes are present in approximately equal proportions in the submucosa and muscle [260]. Heterogeneity with regard to formalin fixation has also been demonstrated in mast cells of the human nose [261], skin [262] and lung [263]. However, the distinction between the cell types is considerably more subtle and less obvious than in the gastrointestinal tract of the rat. Moreover, the subpopulations are no longer confined to particular anatomical areas of the target organ. Thus, the 'mucosal' mast cell of the human large intestine, as defined by its formalin sensitivity, is not restricted to the lamina propria but is observed in great numbers in the submucosa and muscle [260]. Under these conditions, the terms 'mucosal' and 'connective tissue' mast cell must clearly be used with great caution.

1.6.2 Biochemical differences between mast cells

As discussed above, rat CTMC such as those derived from the serosal cavities contain heparin predominantly. In contrast, mast cells obtained from the enzymic dispersion of intestinal mucosa from the same animal contain a form of chondroitin sulphate termed chondroitin sulphate diB [264,265]. The major proteoglycan of human lung mast cells is heparin [257,264] but this is of considerably lower relative molecular mass than that of the rat (60,000 and 650,000 respectively). Moreover, a recent study has shown that these cells also contain chondroitin sulphates E and A [101].

Apparent heterogeneity of neutral proteases has been observed in rat mast cell populations [257,264,266]. Rat CTMC contain a chymotrypsin-like neutral protease termed rat mast cell protease I (RMCP-I) and carboxypeptidase A, an enzyme that cleaves C-terminal aromatic amino acids, whereas rat MMC and those cultured from bone marrow contain another chymotrypsin-like neutral protease named rat mast cell protease II (RMCP-II) [106,267,268]. Although RMCP-I and RMCP-II have substantial homology in their amino acid sequences, antibodies to each of them do not cross react [269]. This difference in neutral protease content also extends to human mast cells in which two enzymes, tryptase (T) and chymase (C) are present. The predominant cell

in the lung and the intestinal lamina propria contains tryptase alone (T mast cell) whereas the predominant cell in the skin and intestinal submucosa contains both enzymes (TC mast cell) [107,270,271].

The T mast cell is thymus dependent since patients with acquired immunodeficiency syndrome and severe combined immunodeficiency syndrome demonstrated a significant decrease in this type of mast cell in the intestine, whereas the TC type of cell was not diminished [272]. This thymus dependency also applies to rat MMC, since interleukin 3 (IL-3) derived from T cells has been demonstrated to promote their growth in vitro [273], and the hyperplasia of intestinal MMC found in response to the nematode Nippostrongylus brasiliensis does not occur in the nude rat [274].

Metabolism of arachidonic acid via the cyclooxygenase and lipoxygenase pathways produces compounds that are highly vasoactive, spasmogenic and chemotactic. However, the relative importance of the two pathways varies from one cell type to another. Rat peritoneal mast cells process arachidonic acid almost exclusively through the cyclooxygenase pathway to form PGD_2 with little or no production of leukotrienes [119]. In contrast, in intestinal MMC from the same animal, leukotrienes in the form B_4 and C_4 are produced along with PGD_2 [121]. Arachidonic acid metabolism in cultures of bone marrow-derived mouse mast cells, which show some of the characteristics of rat MMC, preferentially leads to the synthesis of LTC_4 [275]. The situation in human mast cells is more controversial due to the difficulty in obtaining pure cell preparations but it would now appear that mast cells derived from the lung [216,276], intestine [77] and skin [277] are capable in producing both PGD_2 and leukotrienes.

1.6.3 Functional differences between mast cells

As already discussed in section 1.3, mast cells can be activated by a variety of immunological and non-immunological stimuli. The response to non-immunological stimuli is especially interesting since it provides a striking example of mast cell heterogeneity.

The synthetic polyamine compound 48/80 has often been described as the 'classical mast cell degranulating agent' but is, in fact, highly specific in its action. The polycationic compound is a potent releaser of histamine from rat serosal mast cells [278]. Enzymically dispersed mesenteric, lung and skin mast cells from the same animal show significant reactivity whereas cardiac and intestinal mast cells are totally unresponsive [257,279]. Peritoneal mast cells of the hamster and particularly of the mouse, are much less reactive than those of the rat and tissue mast cells of the guinea pig are refractory to the compound [257,279]. The situation in man is equally complex, enzymically dispersed adenoid, colonic, lung and tonsil mast cells together with human basophils are unresponsive to compound 48/80 [66,76,280,281]. In contrast, this agent is active towards mast cells from the skin and synovial fluid [66,280,282].

Indeed, this pattern of differential responsivity extends to other non-immunological secretagogues and also to agents that modulate histamine secretion from the mast cell; one well documented example is provided by the anti-asthmatic drug disodium cromoglycate (DSCG). DSCG is a potent inhibitor of IgE-mediated histamine release from rat serosal mast cells [257,279,283]. In contrast, the compound is significantly less active against peritoneal mast cells from the hamster and totally ineffective against those from the mouse. The chromone is similarly inactive against tissue mast cells of the guinea pig and intestinal mucosal mast cells of the rat [257,279]. In man, this drug is totally ineffective against the basophil [257,279] but has significant activity towards mast cells isolated from the colonic mucosa [76]. In the lung, DSCG shows limited activity against pulmonary mast cells isolated from the lung parenchyma while being significantly more active towards airway cells recovered by bronchoalveolar lavage [284,285].

1.6.4 Origins of mast cell heterogeneity

The origin of mast cell heterogeneity is currently the subject of intense research; evidence concerning this phenomenon has mainly been derived from studies with rodents. It now appears that both mast cells and basophils originate from multipotential haematopoietic stem cells from the bone marrow [266]. Like neutrophils and eosinophils, basophils complete their differentiation within the bone marrow, then

circulate in the blood, and finally function and die in tissues [24,266,286]. In contrast, undifferentiated mast cell precursors leave the bone marrow, migrate in the blood, invade tissues, and then proliferate and differentiate into mast cells [266,286].

The generation of mast cells from haematopoietic cells was first shown in the mouse by Kitamura and his co-workers in a series of experiments exploiting genetically mast cell-deficient mutant mice and their congeneic normal (+/+) littermates [266,287]. One of the mutants, the WBB6F_1-W/W^V, is virtually devoid of mast cells but can develop tissue mast cell populations if it receives bone marrow cells derived either from its normal counterparts (WBB6F_1-+/+) or from (C57BL/6-bg^J/bg^J) 'beige' mice [287]. Because mast cells derived from beige mice can be recognized on the basis of their giant cytoplasmic granules [266,288], the mast cells that develop in WBB6F_1-W/W^V mice transported with C57BL/6-bg^J/bg^J bone marrow cells can be identified unambiguously as of donor origin [287].

These mutant mice can also be used to demonstrate phenotypic changes between mast cell populations. Nakano et al [289,290] cultured mast cells (BMCMC) from the bone marrow of WBB6F_1-+/+ mice (BMCMC shared some characteristics of rat MMC) and transferred them into the peritoneal cavity of genetically mast cell-deficient WBB6F_1-W/W^V mice. At various times after the intraperitoneal transfer, mast cells were recovered from the peritoneal cavity. 10 weeks after the initial transfer, the recovered mast cells acquired the electron microscopic feature of CTMC [266,289]. Furthermore, the histamine content increased more than 20-fold. Although the starting BMCMC did not stain with berberine sulphate, the recovered mast cells stained with this fluorescent dye. This suggests that BMCMC acquired the ability to synthesize and store heparin proteoglycan after the intraperitoneal transfer [289,290].

The phenotypic change occurs in the opposite direction as well [291]. When purified peritoneal CTMC of WBB6F_1-+/+ mice were cultured in methylcellulose with IL-3 and IL-4, about 25 % of the CTMC formed colonies, all of which contained berberine sulphate-positive and berberine sulphate-negative mast cells. When these mast cells were grown in suspension culture, they generated populations that were 100 % berberine sulphate-negative, and that synthesized predominantly chondroitin sulphate

proteoglycans [266,291]. When these MMC-like cultured mast cells were injected into the peritoneal cavity of $WBB6F_1$-W/W^V mice, the adoptively transferred mast cell population became 100 % berberine sulphate-positive [266,291].

The fate of CTMC derived from $WBB6F_1$-+/+ mice was also investigated in the stomach wall of $WBB6F_1$-W/W^V mice [292]. After the injection of a single CTMC, mast cells appeared both in the mucosa and muscularis propria. Mast cells that appeared in the mucosa showed the biochemical and electron microscopic feature of MMC, whereas the cells that appeared in the muscularis propria showed the feature of CTMC.

In summary, the presence of heterogeneity within mast cells may be explained by their unique differentiation process. Mast cell precursors begin their differentiation after invading particular tissues, the phenotype of the mast cell is then influenced by the tissue microenvironment in which differentiation occurs. However, the mechanism that explains how the phenotype is determined by tissue factors remains to be clarified.

1.7 Aims of this study

As described above, mast cells from different species and even from different tissues within the same animal may exhibit marked variations in their biochemical, histochemical and functional properties. Therefore, it is the aim of the present study to investigate this phenomenon further, paying particular attention to the functional aspects of mast cell heterogeneity. Species heterogeneity will be first studied, followed by some more in depth investigations into the problem of tissue heterogeneity, concentrating solely on the rat and man.

The present project will also extend to human mast cells in different pathological conditions. Since mast cell hyperplasia has been reported to occur in inflammatory bowel diseases (IBD), mast cells from macroscopically normal colonic mucosa of cancer patients will be compared to those obtained from patients suffering from Crohn's disease and ulcerative colitis. Finally, some basic characterizations of human uterine and stomach mast cells will also be reported.

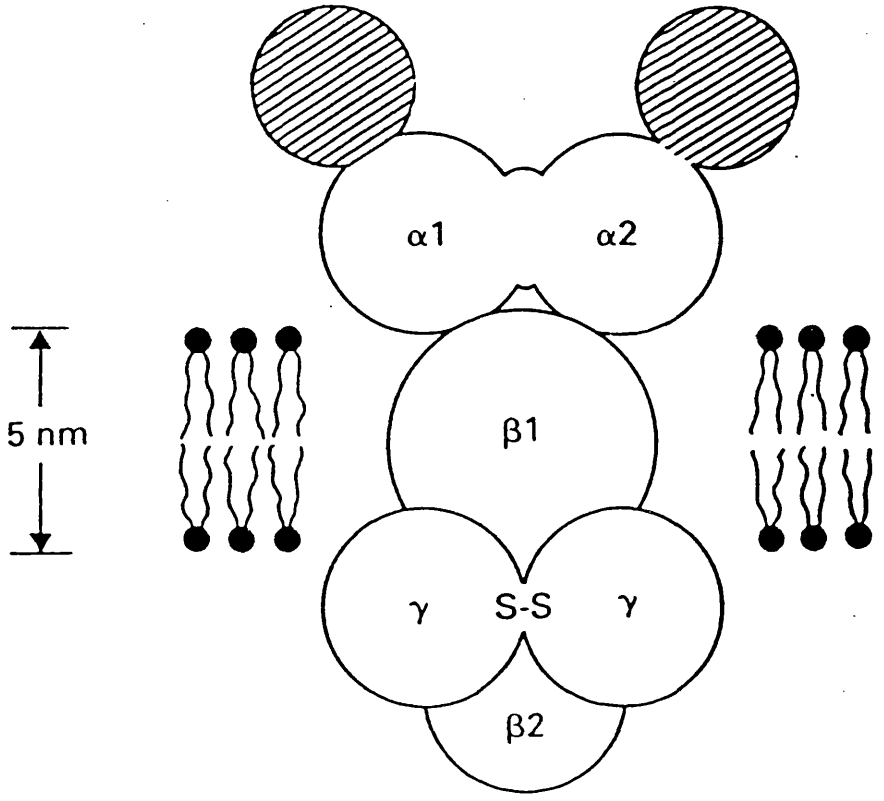


Fig 1.1 Diagram of the high-affinity receptor for IgE, showing the relationships between the subunits in the membrane. Shaded areas represent carbohydrate. Source: H. Metzger, J-P. Kinet, R. Perez-Montfort, V. Rivnay and S.A. Wank: Progress in Immunology, 5, P493, Academic press, New York, (1983).

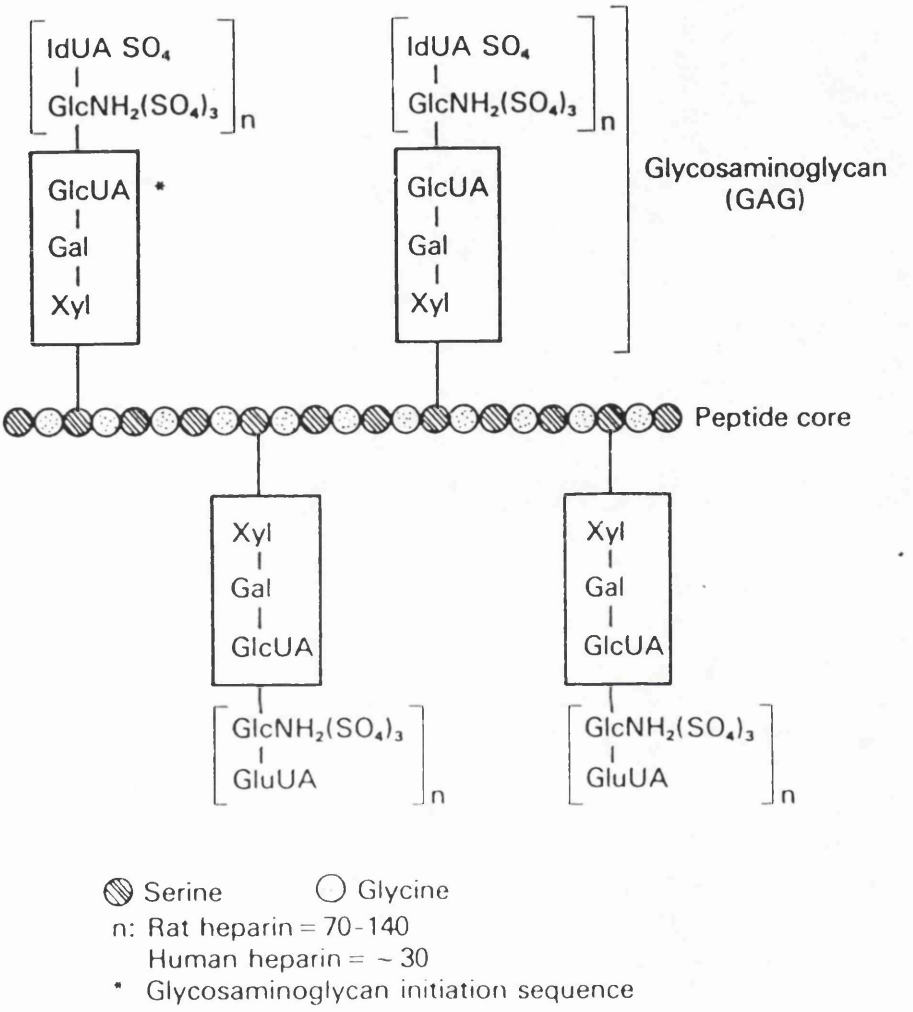


Fig 1.2 Schematic representation of structure of heparin. Xyl = xylose; Gal = galactose; GlcUA = glucuronic acid; IdUA = iduronic acid; $GlcNH_2$ = glucosamine; SO_4 = sulphate. Source: T. Ishizaka: Allergy, Principles and Practice (Eds. Middleton, Reed, Ellis, Adkinson and Yunginger), vol 1, P71, The C.V. Mosby Co., (1988).

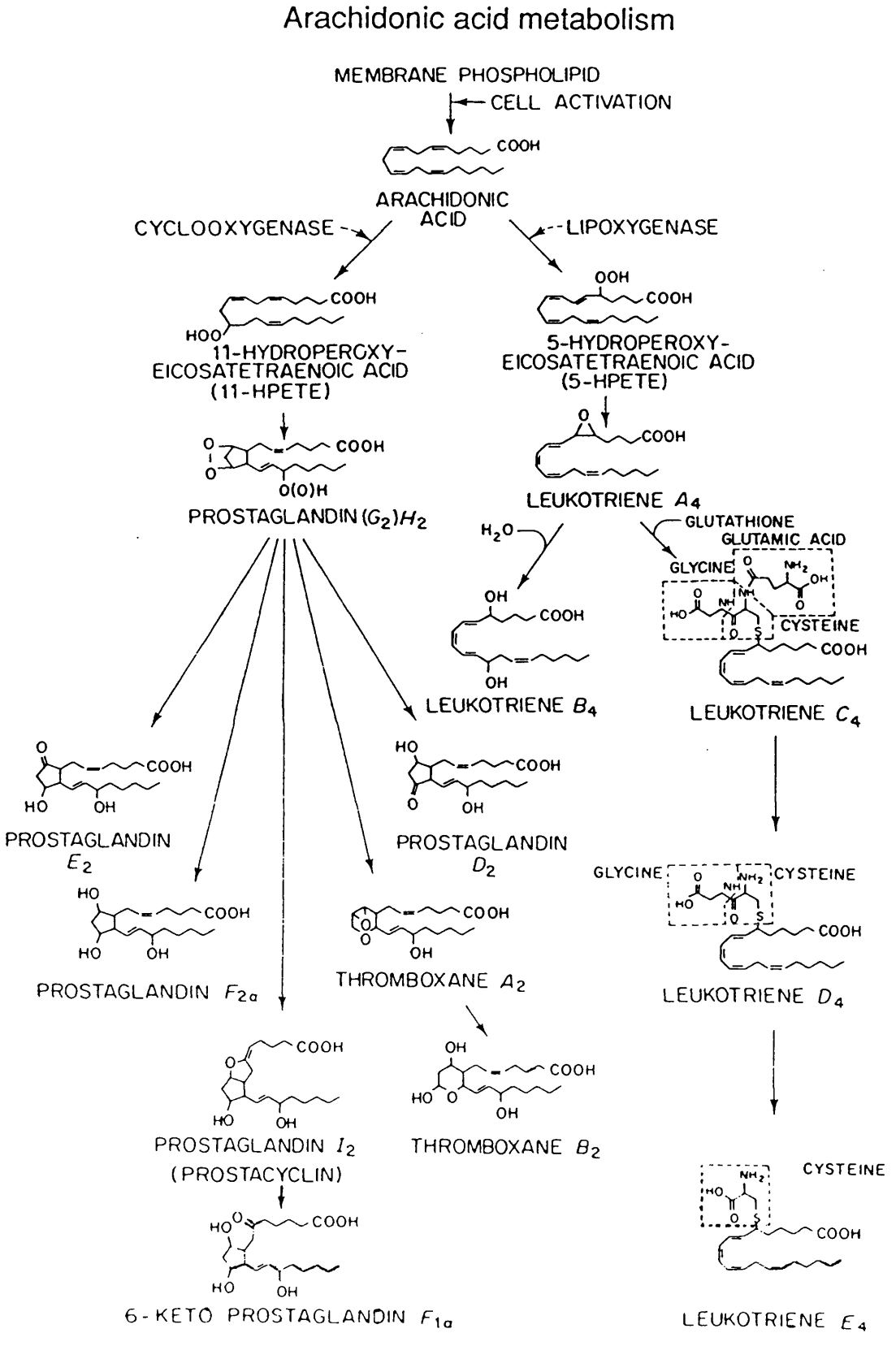

Arachidonic acid metabolism
MEMBRANE PHOSPHOLIPID
CELL ACTIVATION
ARACHIDONIC ACID
CYCLOOXYGENASE
LIPOXYGENASE
11-HYDROPEROXY-EICOSATETRAENOIC ACID (11-HPETE)
5-HYDROPEROXY-EICOSATETRAENOIC ACID (5-HPETE)
PROSTAGLANDIN $(G_2)H_2$
LEUKOTRIENE A_4
H_2O
GLUTATHIONE
GLUTAMIC ACID
GLYCINE
CYSTEINE
LEUKOTRIENE B_4
LEUKOTRIENE C_4
PROSTAGLANDIN E_2
PROSTAGLANDIN D_2
PROSTAGLANDIN $F_{2\alpha}$
THROMBOXANE A_2
LEUKOTRIENE D_4
PROSTAGLANDIN I_2 (PROSTACYCLIN)
THROMBOXANE B_2
6-KETO PROSTAGLANDIN $F_{1\alpha}$
LEUKOTRIENE E_4

Fig 1.3

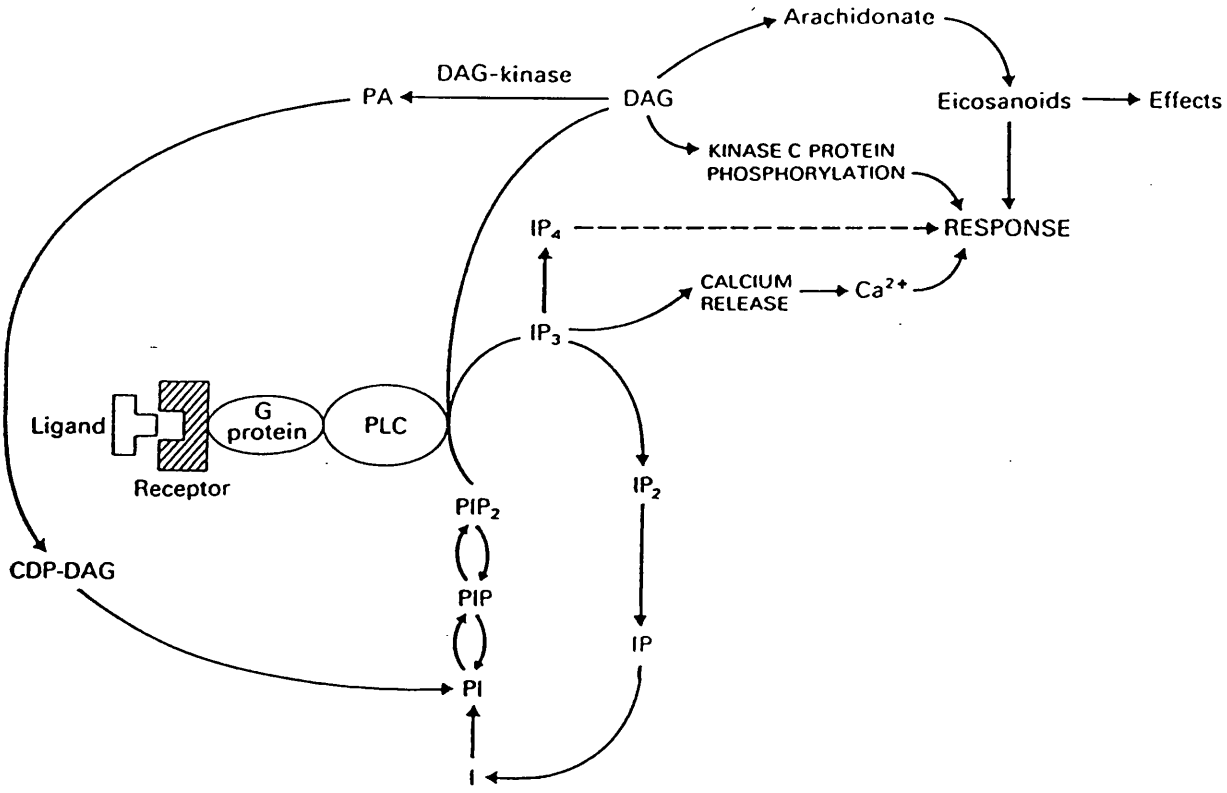


Fig 1.4 Sequence of reactions involved in phosphoinositide breakdown. I = inositol; PA = phosphatidic acid; CDP-DAG = cytidine diphosphodiacylglycerol; DAG = diacylglycerol; PLC = phospholipase C; PIP_2 = phosphatidylinositol-4,5-bisphosphate; PIP = phosphatidylinositol-4-phosphate; PI = phosphatidylinositol; IP_3 = inositol-1,4,5-trisphosphate; IP_3 = inositol-1,4-bisphosphate; IP = inositol-4-phosphate and inositol-1-phosphate; IP_4 = inositol-1,3,4,5-tetrakisphosphate. Source: J.C. Foreman: Textbook of Immunopharmacology (Eds. M.M. Dale and J.C. Foreman), P19, Blackwell Scientific Publications, (1989).

Sequence of reactions involved in phospholipid methylation in rat mast cells

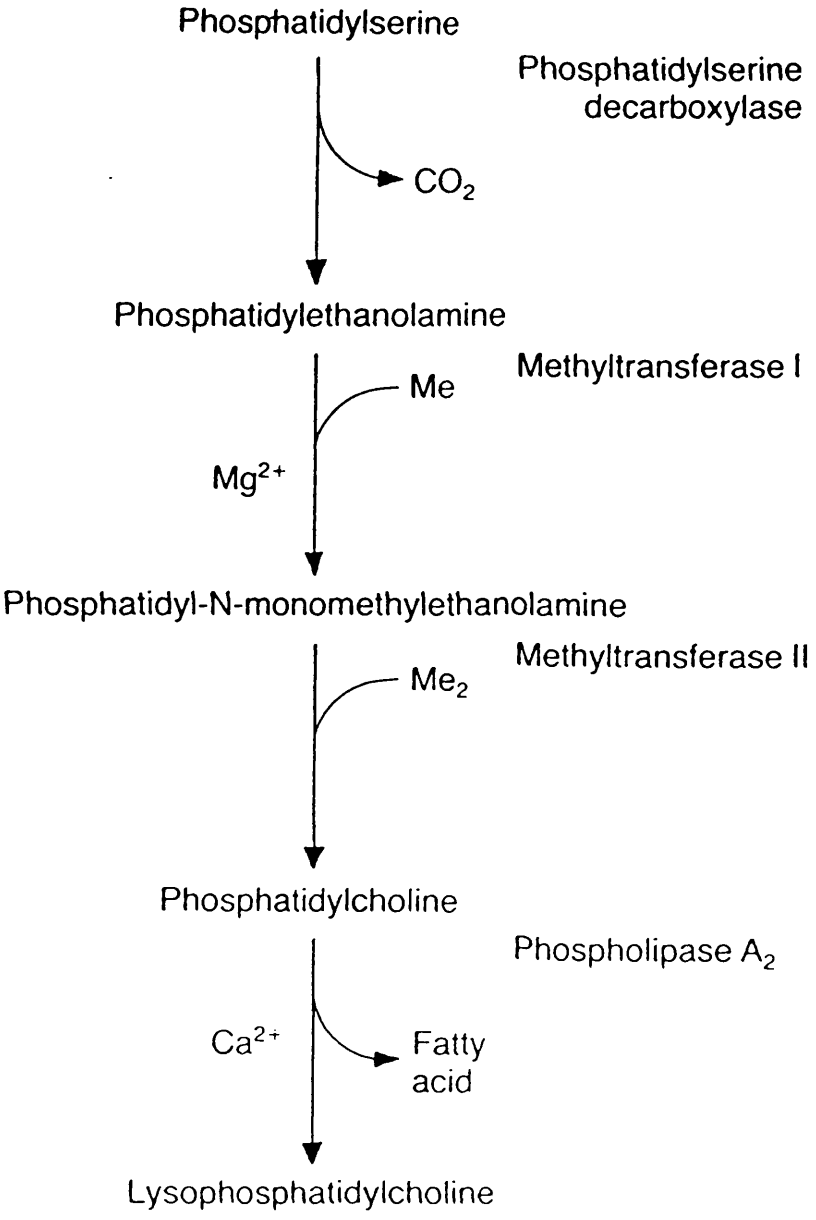


Fig 1.5

Table 1.1 Some properties of mucosal and connective tissue mast cells from the gastrointestinal tract of the rat.

Mucosal mast cells	Connective tissue mast cells
Small, variable shape, sparsely granulated	Large, uniform, densely granulated
Uni- or bilobed nucleus	unilobed nucleus
Soluble granular proteoglycan matrix	Less soluble proteoglycan matrix
Chondroitin sulphate	Heparin
Low content of histamine and 5-hydroxytryptamine	High monoamine content
Metachromasia may be blocked by conventional aldehyde fixation	Metachromasia preserved by aldehyde fixation
Berberine negative	Berberine positive
Stain with alcian blue but do not counterstain with safranin	Counterstain with safranin
Proliferative, non-secretory polyamine response	Secretory polyamine response
Short life span	Long life span
Proliferative response to nematode infection	No proliferative response
IgE in cytosol	No IgE in cytosol
Contain rat mast cell protease II	Contain rat mast cell protease I
Cromoglycate insensitive	Cromoglycate sensitive

CHAPTER TWO

METHODS AND MATERIALS

2.1 Animals

Throughout the present study, Dunkin Hartley guinea pigs (400 - 600 g), Syrian hamsters (50 - 200 g), BKW mice (30 - 50 g) and Sprague Dawley rats (200 - 400 g) of either sex were used. These animals were obtained from closed, random bred colonies kept in the Joint Animal House, University College London. Lung samples from Sus scrofu (great white) pigs (15 - 20 Kg) were supplied by Mr.A. Marath (Westminster Hospital, London).

2.2 Human Subjects

Human colonic, lung, stomach and uterine tissues were provided by surgeons of the Middlesex Hospital (London), University College Hospital (London), St. Bartholomew's Hospital (London) and Elizabeth Garrett Anderson Hospital (London) respectively. Peripheral blood was obtained by venipuncture from normal healthy donors by a qualified medical practitioner.

2.3 Buffers

All experiments (unless otherwise stated) were carried out using modified Tyrode's solution buffered with N-2-hydroxyethyl piperazine-N'-2-ethane sulphonic acid (HEPES) as described below. The pH of all buffers was adjusted to 7.4 by addition of either HCl (3M) or NaOH (4M).

2.3.1 Full HEPES-Tyrode's

NaCl	137.0 mM (8.0 g/l)
Glucose	5.6 mM (1.0 g/l)

KCl	2.7 mM (201 mg/l)
$CaCl_2$	1.0 mM (147 mg/l)
HEPES	10.0 mM (2.9 g/l)
NaH_2PO_4	0.4 mM (62.5 mg/l)

2.3.2 2x Calcium-Tyrode's

Full HEPES-Tyrode's containing $CaCl_2$ at 2x the normal concentration (2.0 mM) or (294 mg/l).

2.3.3 Calcium-free-Tyrode's (CMF)

Full HEPES-Tyrode's in which $CaCl_2$ was omitted.

2.3.4 Glucose-free-Tyrode's

Full HEPES-Tyrode's in which glucose was omitted.

2.3.5 2x EDTA-Tyrode's

CMF-Tyrode's containing EDTA (0.2 mM) or (75 mg/l).

2.4 Isolation of mast cells

2.4.1 Peritoneal mast cells

The method employed for the isolation of peritoneal mast cells was similar for all experimental animals used. Essentially, the animal was first anaesthetised with nitrous oxide, allowed to expire under the anaesthetic, and the abdominal skin removed. Full Tyrode's containing heparin (5 units/ml) was injected into the peritoneal cavity (15 ml per rat, 10 ml per hamster and 5 ml per mouse). Gentle massage (2 min) was applied to the animal and the abdomen was cut open along the midline. Peritoneal cells were recovered using a plastic pipette and were collected in a plastic test tube. Any

sample heavily contaminated with blood was discarded. The cells were then pelleted by centrifugation (100 g, room temperature (RT), 2 min), washed twice in the appropriate buffer and then used for functional studies without further purification.

2.4.2 Rat pleural mast cells

Pleural cells were usually recovered after isolation of peritoneal cells from the same animal. Heparin-Tyrode's (8 ml) was injected into the pleural cavity through the diaphragm and the animal was massaged for a further 2 minutes. The rib-cage of the animal was then cut open and the pleural cells recovered using a plastic pipette. The cells were washed and treated as described in 2.4.1 for peritoneal mast cells.

2.4.3 Rat and guinea pig mesenteric and lung mast cells

The animal was anaesthetised as before. The mesentery was removed from the small intestine and dissected free of any attached lymph nodes. The lungs were removed from the chest cavity and dissected free of major airways. Each tissue was rinsed in Full Tyrode's and then cut into small fragments (1-2 mm) with scissors and an automated (McIlwain) tissue chopper. This was followed by digestion (37 °C, 60 min with stirring) in Full Tyrode's containing 20 % foetal calf serum (FCS) and collagenase (120 units/ml). At the end of the incubation, the digested tissue was further disrupted by expression through a 20 ml syringe and the dispersed cells were isolated by filtration through nylon gauze. The cells were recovered by centrifugation (150 g, 4 °C, 3 min) and washed once in Full Tyrode's containing 10 % FCS and twice in Full Tyrode's. The cell suspension was then used for functional studies without further purification.

2.4.4 Rat intestinal mast cells

The animal was killed under anaesthesia as before. The whole small bowel (from stomach to colon) was removed. The bowel was flushed clean of faecal matter with Full Tyrode's by the use of a 20 ml syringe. The tissue was then cut into fragments of about 3 cm long and these were opened longitudinally. The mesentery and the

Peyer's patches were removed along with any loose mucus and the fragments were cut further into thin strips of about 2 mm wide. The strips were washed in pre-warmed CMF-Tyrode's (100 ml, 37 °C, 3x10 min with stirring) to remove any remaining mucus followed by digestion in Full Tyrode's containing 20 % FCS and collagenase (40 units/ml) (100 ml, 37 °C, 2x60 min with stirring).

At the end of each digestion, the dispersed cells were filtered through nylon gauze, passed through a wool column and recovered by centrifugation (150 g, RT, 3 min). The wool column consisted of loose nylon scrubbed wool (1 g) packed in a 10 ml syringe. After the second digestion, the tissue was subjected to further disruption by expression through a 20 ml syringe and the cells were recovered as before. Cells extracted were resuspended in Full Tyrode's containing 10 % FCS and kept at 37 °C. The three portions of cells were pooled, washed twice in Full Tyrode's and were then used for functional studies without further purification.

2.4.5 Human colonic mast cells

Macroscopically normal human colonic tissue was recovered following surgery for colonic carcinoma while abnormal tissue was obtained from surgery for Crohn's disease and ulcerative colitis. The tissue was divided into two portions by blunt dissection; one containing the mucosa while the other portion included the submucosa plus the underlying muscle layers.

For each portion, the tissue was washed in Full Tyrode's (37 °C, 2x10 min, with stirring) to remove faecal matter and mucus. It was then cut into small fragments (1-2 mm) with scissors and an automated tissue chopper. This was followed by digestion in Full Tyrode's containing BSA (1 mg/ml) and collagenase (120 units/ml) (25 ml/g tissue, 37 °C, 2x60 min, with stirring). Thereafter, colonic cells were recovered and used in the same way as rat intestinal cells with the exception that no passage through a wool column was required.

2.4.6 Human gastric mucosal mast cells

Macroscopically normal human stomach tissue was recovered following surgery for gastric carcinoma. The mucosa was first separated from the underlying muscle layers by blunt dissection and thereafter treated with the same procedure as described in section 2.4.5 for the isolation of human colonic mast cells.

2.4.7 Human lung parenchymal mast cells

Macroscopically normal human lung tissue was recovered following surgery for bronchial carcinoma and dissected free of major airways and blood vessels. Thereafter, the lung tissue was treated with the same procedure as described in section 2.4.5 for the isolation of human colonic mast cells.

2.4.8 Pig lung mast cells

Lung tissue was obtained from Sus scrofu (great white) pigs (15-20 Kg). Anaesthesia was induced by ketamine and maintained by pancuronium, fentanyl, nitrous oxide and oxygen. A median sternotomy was performed and a piece of lung (ca. 4x4 cm, 3 g) was resected from the diaphragmatic lobe [293]. The tissue was dissected free of major airways and blood vessels and then treated with the same procedure as described in section 2.4.5 for the isolation of human colonic mast cells.

2.4.9 Human uterine myometrial mast cells

Macroscopically normal human uterine tissue was recovered following surgery for various uterine dysfunctions including cancer and fibrosis. The myometrium was first separated from the endometrium by blunt dissection and thereafter the tissue was treated with the same procedure as described in section 2.4.5 for the isolation of human colonic mast cells.

2.4.10 Human basophils

Venous blood was obtained from healthy human volunteers and heparin (25 units/ml) was added to prevent coagulation. Dextravan 70 containing glucose (30

mg/ml) was mixed with the blood sample in a ratio of 1:4 and the mixture left to stand (60-90 min, RT). Leukocytes were recovered from the top plasma layer by centrifugation (150 g, RT, 4 min), washed twice in Full Tyrode's and then used for functional studies without further purification.

2.4.11 Cultured mouse mast cells

Cultured mouse mast cells, grown in a conditioned medium containing interleukin 3 (IL3), were generously donated by Dr.T. Lamas, Department of Zoology, University of Nottingham. The cells were washed and treated as described in 2.4.1 for peritoneal mast cells.

2.5 Mast cell characterization

2.5.1 Cell counts and viability

The total number of nucleated cells and their viability in any cell suspension was evaluated using the Trypan blue exclusion test (0.1 %). The cells were counted in an improved Neubauer haemocytometer.

2.5.2 Cell fixation and staining

Cytocentrifuge smears (a minimum of 1,000 cells per slide) were made and air-dried. They were fixed in Carnoy's solution (30 min) or formol saline (24 hr) and then stained as follows:

1. Distilled water	(1 min)
2. Alcian blue (0.1 % in 0.7 M HCl)	(30 min)
3. HCl (0.7 M)	(5 min)
4. Safranin O (0.5 % in 0.125 M HCl)	(5-7 min)
5. Distilled water	(1 min)
6. Mount in Canada Balsam	

Carnoy's solution was prepared by mixing absolute ethanol, chloroform and glacial acetic acid in the ratio 6:3:1, v/v. Formol saline was prepared by adding formalin (10 %, v/v) to 0.9 % saline.

Assessment of formaldehyde sensitivity in any given cell preparation was carried out as follows. A total of fifty high power microscope fields were counted in both fixation groups using a Wild Heerbrugg optical microscope. Counts were made at 100x magnification using a calibrated eyepiece (15x GK, Wild Heerbrugg). The number of mast cells was then expressed as a percentage of the total nucleated cell number in each fixation group and the percentage of formaldehyde sensitive, and therefore insensitive mast cells, was calculated by dividing the percentage of mast cells observed in the formaldehyde group by the percentage in the Carnoy's group. Similarly, the percentage of safranin positive mast cells was evaluated by dividing the number of counterstained mast cells by the observed total (alcian blue positive + safranin positive) in the Carnoy's group.

2.5.3 Histamine content

The number of mast cells in a given suspension of the rat peritoneal fluid was assessed by staining an aliquot of the suspension with toluidine blue (15 min, 0.005 %, w/v) and counting the number of stained cells by using an improved Neubauer haemocytometer. The cell suspension was then assayed for histamine and the result compared with known standards to determine the histamine content.

For tissue mast cells, an alternative method was employed to overcome the unsatisfactory staining of these cells by toluidine blue. The number of nucleated cells in a given suspension was first determined by the Trypan blue exclusion test (0.1 %). The percentage of mast cells was then assessed by counts performed on stained cytocentrifuge smears. The suspension was assayed and the results compared to known standards as described above.

2.6 Histamine and eicosanoid release experiments

2.6.1 Histamine release from isolated mast cells and basophils

Isolated mast cells and basophils were aliquoted into disposable polystyrene test tubes (peritoneal : 500 µl; others : 200 µl) and were left to equilibrate in a water bath (37 °C, 5 min). A solution of the releasing agent was then added and the reaction allowed to proceed for 10 min (basophils: 45 min). The reaction was terminated by the addition of ice-cold Tyrode's solution (peritoneal : 1.5 ml; others : 800 µl) with the exception of rat intestinal mast cells in which pre-warmed Tyrode's solution (37 °C) was required. The cells were immediately separated from the supernatant by centrifugation (peritoneal : 100 g, 4 °C, 2 min; others : 150 g, 4 °C, 3 min). The resultant cell pellets were resuspended in Tyrode's solution (peritoneal : 2 ml; others: 1 ml) and then boiled for 10 min (manual assay) or were treated with 70 % perchloric acid (final concentration 0.4 M) (automated assay) to release residual histamine. Histamine was determined in both the supernatant and cell pellet and the release was expressed as a percentage of the total amount of the amine originally present in the cells. Hence:

$$\text{Histamine release } (H_R)\ (\%) = 100\ \{\ (H_S) / (H_S + H_C)\ \}$$

where H_S represents the amount of histamine in supernatant and H_C the corresponding amount remaining in the cell pellet. In all experiments, values were corrected for the spontaneous release occurring in the absence of any stimulus.

2.6.2 Histamine release from isolated mast cells and basophils: effect of basic secretagogues

In experiments designed to investigate the effect of polybasic compounds and neuropeptides, isolated mast cells and basophils were first preincubated with the protease inhibitor captopril (400 µl/ml, 37 °C, 5 min) before the addition of the releasing agent. Thereafter, the reaction was terminated and histamine contents determined as before.

2.6.3 Histamine release from isolated mast cells and basophils: effect of calcium

In experiments designed to investigate the effect of calcium, isolated mast cells and basophils (250 µl in CMF-Tyrode's) were first aliquoted into disposable polystyrene test tubes containing an equal volume of appropriately modified Tyrode's solution. This solution contained either CMF, EDTA (200 µM) or Full Tyrode's (containing calcium at 2x the normal concentration). The cells were allowed to equilibrate in the appropriate media (37 °C, 5 min) before the addition of the releasing agent (dissolved in CMF-Tyrode's). Thereafter, the reaction was terminated and histamine contents determined as before.

2.6.4 Histamine release from isolated mast cells and basophils: effect of metabolic inhibitors

In experiments designed to investigate the metabolic requirements for histamine release, isolated mast cells and basophils were first preincubated (37 °C, 20 min) in glucose-free-Tyrode's containing 2-deoxyglucose (5 mM) and antimycin A (1 µM). The cells were then challenged with the appropriate releasing agent (dissolved in glucose-free-Tyrode's). Thereafter, the reaction was terminated and histamine contents determined as before.

2.6.5 Prostaglandin D_2 (PGD_2) and leukotriene C_4 (LTC_4) release from isolated human colonic mast cells

Isolated human colonic mast cells (200 µl) were aliquoted and equilibrated as outlined in 2.6.1. A solution of the releasing agent was then added and the reaction allowed to proceed for 20 min. The reaction was terminated by the addition of ice-cold Tyrode's solution (800 µl) and immediately centrifuged (150 g, 4 °C, 3 min). Aliquots (2x250 µl) of the supernatant were taken from each sample and pipetted into Eppendorf tubes. They were snap-frozen in liquid nitrogen and kept frozen at -85 °C until required for PGD_2 and LTC_4 assays (sections 2.8 and 2.9). The rest of the supernatant (500 µl) was transferred into clean polystyrene tubes and made up to a final volume of 1 ml. Cell pellets were resuspended in Tyrode's solution to a final volume of 2 ml. The histamine contents of both supernatants and cell pellets were then determined as before.

2.6.6 Kinetics of histamine and eicosanoid release

The general procedure was identical to that set out in 2.6.1 and 2.6.5. Cells were incubated with the appropriate stimulus for a series of pre-selected times and the reactions terminated as before.

2.6.7 Inhibition of histamine release

Isolated mast cells and basophils were aliquoted and equilibrated as before. A solution of the inhibiting agent was added for a defined period of preincubation before the addition of the stimulus with the exception of zero preincubation in which the inhibitor and stimulus were added together. The reaction was allowed to proceed for 10 min (basophils: 45 min) and then terminated as before. The result was expressed in terms of the percentage inhibition of the control release (release in the absence of the inhibitor). Hence:

$$\text{Inhibition } (H_I)\ (\%) = 100\ \{\ (R_C - R_I) / (R_C)\ \}$$

where R_C represents the control release and R_I the release in the presence of the inhibitor.

2.7 Histamine assay

2.7.1 Manual assay

The basis of the histamine assay was first described by Shore et al [294]. It is based on the reaction between histamine and o-phthaldialdehyde (OPT) under alkaline conditions. The resultant condensation product (fig 2.1) is highly fluorescent and can be measured by using a commercially available spectrophotometer (Perkin Elmer LF 5B). The procedure first involved the addition of NaOH (1 M, 267 µl) to each sample (2 ml) followed by OPT (1 % in methanol, 100 µl). The mixture was mixed immediately on a vortex mixer and allowed to react for 4 min. The reaction was terminated by addition of HCl (3 M, 133 µl). The fluorescence generated is measured

on the spectrophotometer using an excitation wavelength of 360 nm and an emission wavelength of 440 nm. The limit of the sensitivity from the machine is ca 5 ng/ml of histamine.

2.7.2 Automated assay

For samples other than those of peritoneal mast cells, the histamine contents were assayed in a commercial autoanalyser (Technicon Autoanalyser II). To prepare the samples for analysis, perchloric acid was added to a final concentration of 0.4 M (to release residual histamine and to precipitate any proteins that might be present). The samples were mixed and centrifuged (200 g, RT, 15 min).

After introduction into the autoanalyser, the samples were made alkaline and the histamine extracted into salt saturated butanol. The organic phase was separated, washed once in a less alkaline medium, made less polar by the addition of n-heptane and the histamine back-extracted into dilute HCl. The amine was allowed to react with OPT under alkaline conditions and the adduct generated was stabilised by acidification. The fluorescence was recorded by a chart recorder. The limit of the sensitivity from the machine is ca 0.5 ng/ml of histamine.

2.8 Leukotriene C_4 (LTC_4) assay

LTC_4 levels were measured by radioimmunoassay (RIA) using a commercially available assay kit (Amersham). The assay is based upon competition between unlabelled leukotriene C_4 and a fixed quantity of tritium-labelled leukotriene C_4 for binding to a limited quantity of an antibody which has high specificity and affinity for peptido-leukotrienes. The antibody is quite specific for LTC_4 but there is cross-reactivity with LTD_4 (64 %), LTE_4 (64 %) and to the 11-trans isomers of LTD_4 (30 %) and LTE_4 (24 %).

In brief, a set of LTC_4 standards (Amersham) and the experimental samples (100 µl) were pipetted into appropriately labelled polypropylene tubes (12x75 mm). This was followed by the addition of the ^3H tracer (100 µl), peptido-leukotriene specific

antiserum (100 µl) and assay buffer (100 µl). Along with the samples and standards, a total count (TC) tube (100 µl tracer, 100 µl antiserum, 200 µl buffer), a zero standard (B_0) tube (100 µl tracer, 100 µl antiserum, 200 µl buffer) and a non specific binding (NSB) tube (100 µl tracer, 300 µl buffer) were also prepared. All tubes were mixed thoroughly and were left to incubate overnight at 4 °C. The next day, a dextran-coated charcoal solution (500 µl) was added to each tube with the exception of the TC tube in which assay buffer (500 µl) was used instead. The tubes were mixed immediately on a vortex mixer, left to stand in an ice-bath for 15 min and were then centrifuged (250 g, 4 °C, 15 min). The supernatants were decanted into scintillation vials. Scintillant (Optiphase Safe LKB, 5 ml) was added into each vial, mixed thoroughly, and the radioactivity measured in a β-scintillation counter (Packard Model 3255 Tris-carb liquid Scintillation Spectrometer, 4 min). A standard curve was constructed from which LTC_4 contents in the samples could be determined. Samples were diluted if necessary to be within the range of the standards. The sensitivity range of the RIA assay for LTC_4 is 15-800 pg/ml.

2.9 Prostaglandin D_2 (PGD_2) assay

A commercially available RIA assay kit (Amersham) was again used for the measurement of PGD_2 levels. The principle and the preparation of standards and experimental samples was identical to that listed in section 2.8 for LTC_4. The antibody is quite specific for PGD_2 but there is cross-reactivity with PGJ_2 (7 %), $PGF_{2\alpha}$ (<1 %), PGE_2 (<1 %), PGA_2 (<1 %) and thromboxane B_2 (<1 %). The sensitivity range of the RIA assay for PGD_2 is 5-200 pg/ml.

2.10 Active sensitization

2.10.1 Sensitization of rats with Nippostrongylus brasiliensis

Sprague Dawley rats (ca 200 g) were sensitized by the subcutaneous injection of third stage larvae (L_3, 2500) of Nippostrongylus brasiliensis in sterile physiological saline (SPS, 0.2-0.3 ml). The larvae were obtained either from faecal cultures of previously infected rats as described in section 2.10.2 or were generously provided by

Mr. D. Pedley, Department of Agricultural Zoology, University of Leeds. The rats were ready for use 21 days post injection and remained sensitized for another 3-4 weeks.

2.10.2 Preparation of third stage larvae of Nippostrongylus brasiliensis

Faeces were collected from the rats 6-8 days after the injection of 2500 L_3 larvae. The faeces were moistened and ground with an equal weight of activated charcoal until a mash was formed. The mixture was then transferred to petri-dishes (fig 2.2), and kept in the dark in an incubator (25 °C) for between 7-21 days. To isolate the L_3 larvae, the contents in each petri-dish were poured onto a glass funnel lined with two layers of gauze interleaved with one layer of lens tissue (fig 2.3). The larvae were allowed to migrate downwards, through warm tap water into a graduated test tube (60-90 min). The resultant larvae were washed 3-4 times with SPS, counted, and finally resuspended in an appropriate volume for injection.

2.10.3 Preparation of secretory allergen

Nippostrongylus brasiliensis secretory allergen was prepared using the method of Keller [295]. In brief, rats were injected with 5000 L_3 larvae as described in section 2.10.1, and were sacrificed 6-8 days post injection. The abdomen was opened and the whole small bowel removed. The gut was cut open along its midline with scissors and then laid on top of the apparatus for L_3 larvae isolation (fig 2.4), omitting the lens tissue and filled with pre-warmed SPS. The worms were allowed to migrate downwards into the graduated test tube (60-90 min), followed by repeated washings (5 times) and finally incubated with SPS (10x worm volumes, 37 °C, 5 hr, with gentle agitation). The resultant supernatant containing the allergen (1 worm equivalent (W.E.) per µl as defined by Ogilvie [296]) was aliquoted and stored at -20 °C until required. The life cycle of Nippostrongylus brasiliensis is shown in fig 2.5.

2.11 Preparation of rat antiserum

Pooled reaginic antiserum was prepared by sensitization of rats with Nippostrongylus brasiliensis essentially according to the method of Ogilvie [296].

Animals were given a primary subcutaneous injection of larvae (L_3, 2500) and reinfected (L_3, 2000) 28 days later. After a further 8 days, they were lightly anaesthetised with ether and bled from the neck. The blood was allowed to clot (37 °C, 1 hr then 4 °C, overnight) and the serum recovered by centrifugation (200 g, 4 °C, 10 min) and heat inactivated (56 °C, 30 min). The recovered serum was aliquoted and stored at -20 °C until required.

2.12 Passive sensitization of mast cells

2.12.1 Rat mast cells

Rat serosal and tissue mast cells were passively sensitized by incubation (37 °C, 2 hr) with gentle agitation in a two-fold dilution of the antiserum (see section 2.11). They were then washed three times with Tyrode's solution and were resuspended in an appropriate volume of buffer for experimental use.

2.12.2 Human mast cells

Human mast cells from dispersed colonic, lung, stomach and uterine fragments were suspended in heparin-Tyrode's solution containing BSA (0.1 %). High IgE titre serum (generously provided by Mr.B. Hudspith, Middlesex Hospital, London) was added to achieve a final concentration of 250 iu/ml of serum. The suspensions were incubated (37 °C, 2 hr then 4 °C, overnight), washed three times with Tyrode's solution and resuspended in the required volume of buffer.

2.13 Use of phosphatidylserine

The phospholipid phosphatidylserine (PS) was obtained commercially as a solution (20 mg/ml) in chloroform / methanol (3:1, v/v). To prepare an aqueous suspension for experimental use, an aliquot (25 µl) was pipetted into a glass test tube and the solvent evaporated under a stream of dry, oxygen-free nitrogen. At the same time, the glass tube was rotated gently to obtain an even coating of the lipid on its walls. CMF-Tyrode's solution (500 µl) was added and the suspension mixed thoroughly on a vortex

mixer. The lipid was added to cells 2 min before the addition of secretagogues, to obtain a final concentration of 15 μg/ml.

2.14 Materials

2.14.1 Immunologically directed secretagogues and lectins

Rabbit antiserum to human IgE (anti-human IgE)	Dako
Sheep antiserum to rat IgE (anti-rat IgE)	ICN
Sheep antiserum to rat IgG (anti-rat IgG)	Miles Laboratories
Concanavalin A (Jack bean)	Sigma, London
Wheat germ lectin (Triticum vulgaris)	Sigma, London

2.14.2 Other secretagogues

Acetylcholine	Sigma, London
Compound 48/80	Sigma, London
Chlortetracycline	Sigma, London
Dextran (MW 110,000)	Fisons
Dimaprit dihydrochloride	Gift from Dr.J. Skidmore, SmithKline and Beecham
Formyl-met-leu-phe	Sigma, London
Human gastrin	Sigma, London
Impromidine trihydrochloride	Gift from Dr.J. Skidmore, SmithKline and Beecham
Ionomycin	Calbiochem
Ionophore A23187	Calbiochem
Ionophore bromo A23187	Calbiochem
Poly-L-arginine.HCl (MW 70,000)	Sigma, London
Poly-L-lysine.HBr (MW 70,000)	Sigma, London

Polymyxin B sulphate	Sigma, London
Substance P (SP)	Peninsula
$SP_{1-4}(CH_2)_{11}CH_3$	Gift from Dr.H. Repke, Academy of Drug Research, Berlin, GDR
Triton X-100	Sigma, London
Tween-20	Sigma, London

2.14.3 Compounds that inhibit histamine release

Chrysin	Sigma, London
Cimetidine	Gift from Dr.J. Skidmore, SmithKline and Beecham
Dibutyryl cAMP	Sigma, London
Dimaprit dihydrochloride	Gift from Dr.J. Skidmore, SmithKline and Beecham
Disodium cromoglycate	Gift from Mr.P. Sheard, Fisons
Isobutyl-methylxanthine	Sigma, London
Isoprenaline (bitartrate)	Sigma, London
LU 48953	Gift from Prof.L. Lenke, Knoll
MY 1250	Gift from Dr.M. Hammond, Bayer
Nedocromil sodium	Gift from Mr.P. Sheard, Fisons
Quercetin	Sigma, London
Ranitidine	Gift from Dr.J. Skidmore, SmithKline and Beecham
Salbutamol (sulphate)	Gift from Dr.D.E. Bays, Glaxo
Theophylline	Sigma, London

2.14.4 Materials for buffers

Calcium chloride	Hopkins and Williams
Ethylenediaminetetraacetic acid (EDTA)	BDH Chemicals
Glucose	BDH Chemicals

HEPES	BDH Chemicals
Hydrochloric acid	Fisons
Potassium chloride	Fisons and BDH Chemicals
Sodium chloride	Fisons
Sodium dihydrogen orthophosphate	Hopkins and Williams
Sodium hydroxide	BDH Chemicals

2.14.5 Materials for fixation and staining

Absolute alcohol (99.8 %)	James Burrough (F.A.D.) Ltd
Alcian blue	BDH Chemicals
Chloroform	BDH Chemicals
Formalin	Hopkins and Williams
Glacial acetic acid	BDH Chemicals
Microscope slides	BDH Chemicals
Safranin O	BDH Chemicals
Toluidine blue	BDH Chemicals
Trypan blue	BDH Chemicals

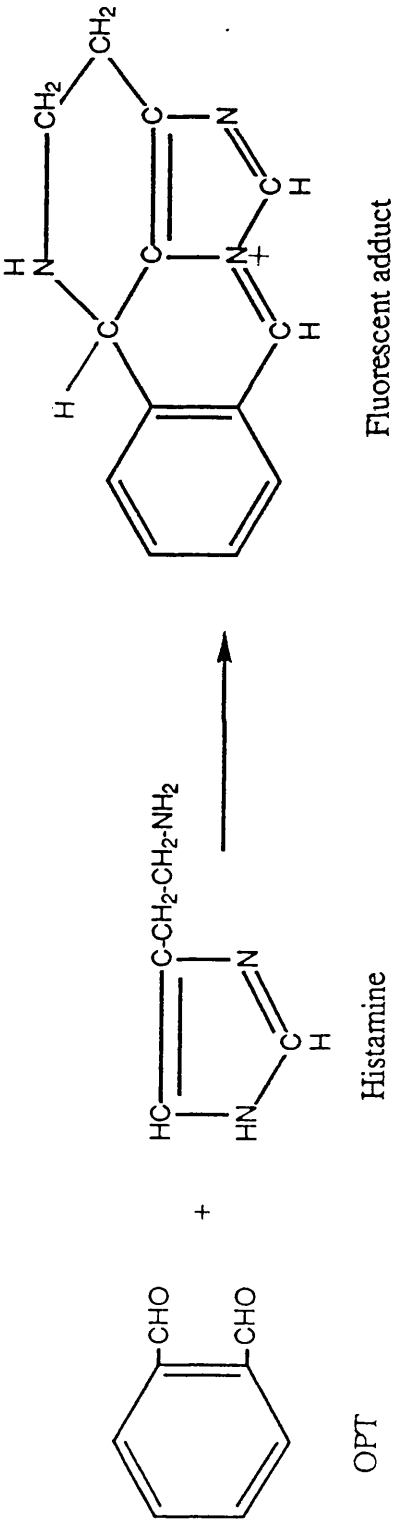
2.14.6 Other materials

Activated charcoal (particle size: 0.85-1.70 mm)	BDH Chemicals
Bovine serum albumin (BSA)	Sigma, London
Butan-1-ol	Fisons
Captopril	Squibb
Collagenase (Type 1A)	Sigma, London
Dextravan 70	Fisons
Dimethylsulphoxide (DMSO)	Hopkins and Williams
Foetal calf serum (FCS)	Gibco Biocult
Heparin	C.P. Pharmaceuticals
n-Heptane	Fisons
Lens tissues	Whatman

Methanol	BDH Chemicals
Nitrous oxide	British Oxygen Company
Nylon scrubbed wool	Fenwal Laboratories
Perchloric acid (72 %)	May and Baker
Phosphatidylserine	Sigma, London and Lipid Products
o-Phthaldialdehyde (OPT)	Sigma, London
Triton X-405	Sigma, London

2.15 Statistical analysis

All values given in this thesis are mean ± s.e.mean for the number (n) of experiments performed. The points on the graphs are the means from the number (n) of experiments noted and the vertical bars represent s.e.means. Statistical analysis of results was generally carried out using the student's t-test for the difference of two independent means. In addition, in appropriate cases, a paired t-test for related measures was performed. Values of $P < 0.05$ were considered to be significant. Denotations by *, ** and *** represent significance levels of $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.



Reaction of histamine with OPT

Fig 2.1

Preparation of third stage larvae

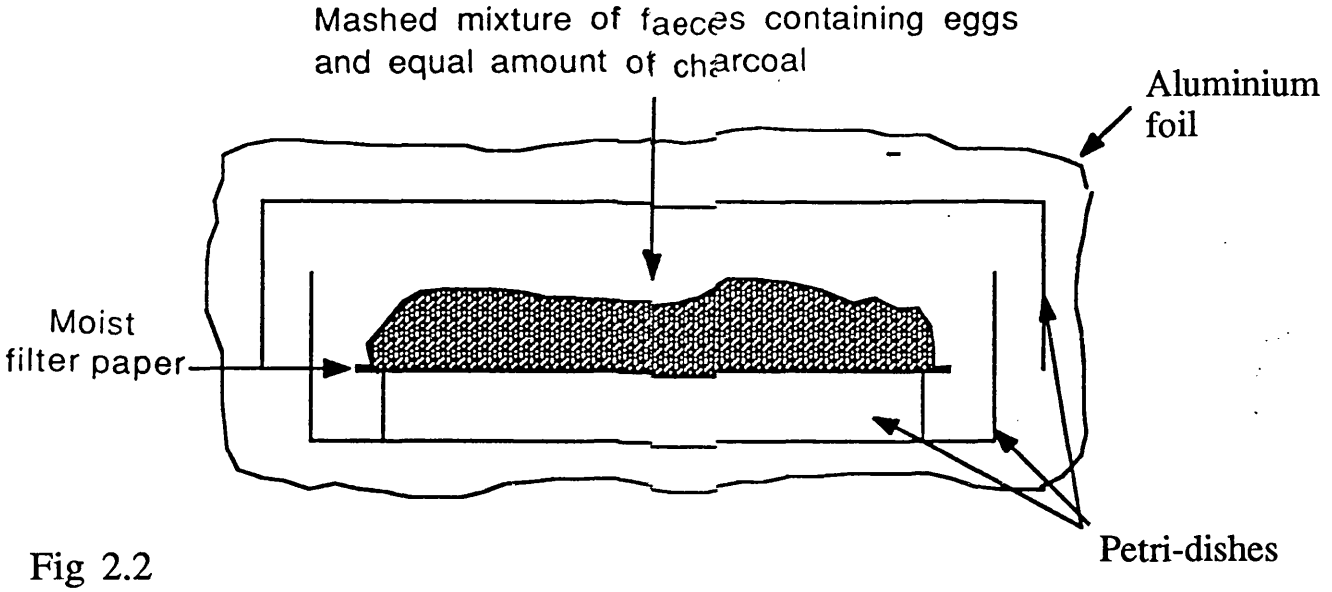


Fig 2.2

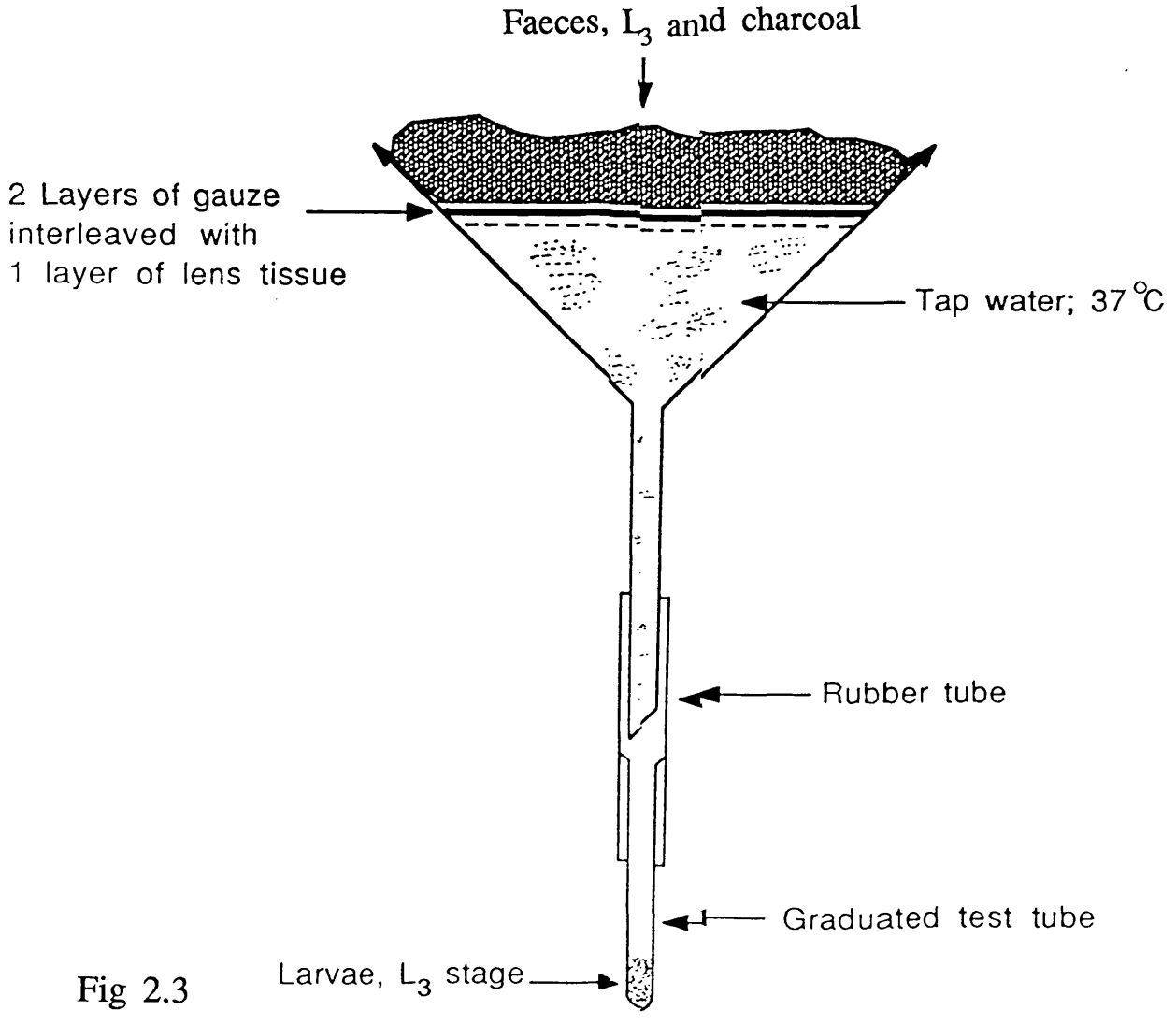


Fig 2.3

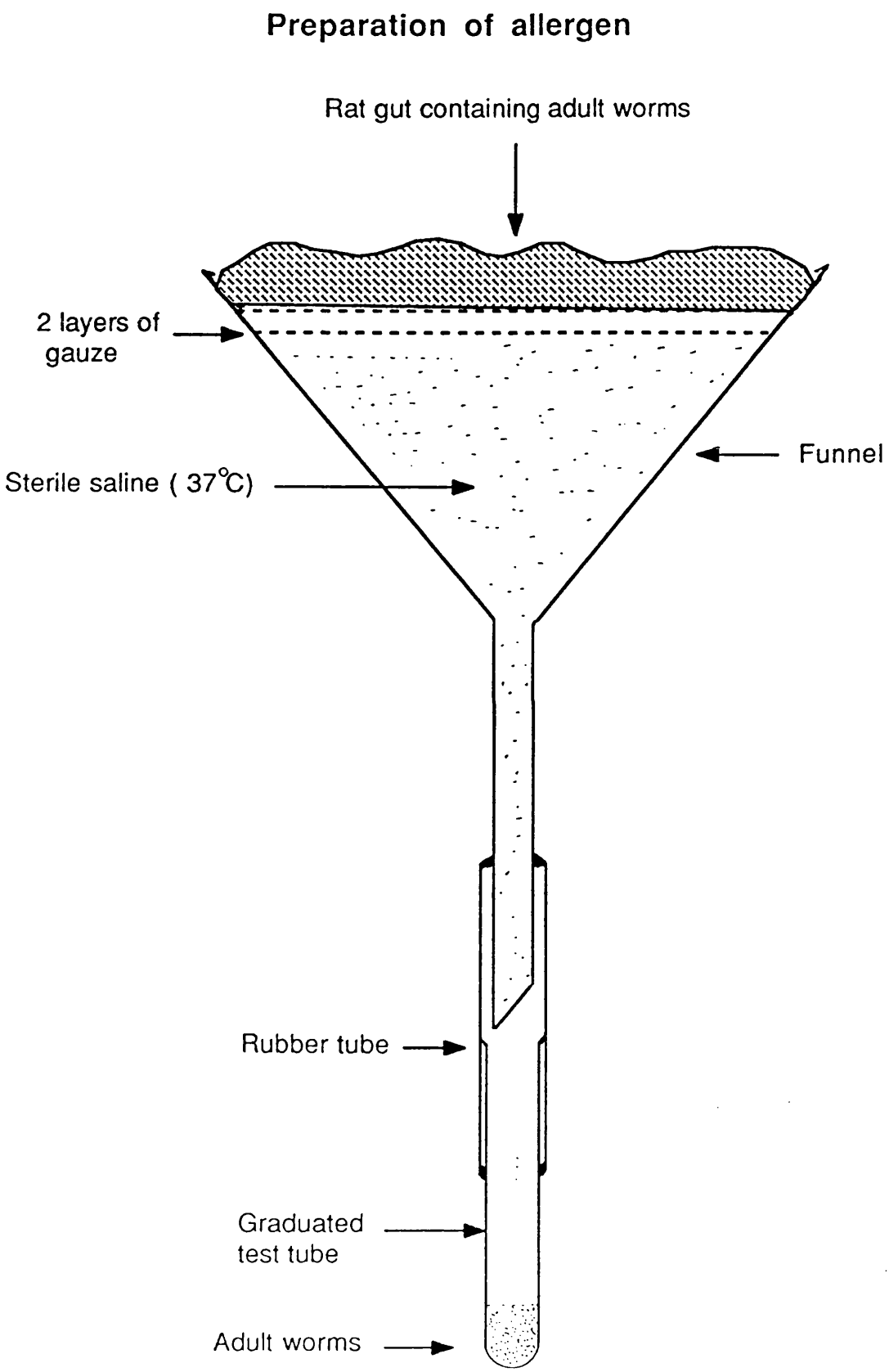

Preparation of allergen
Rat gut containing adult worms
2 layers of gauze
Funnel
Sterile saline (37°C)
Rubber tube
Graduated test tube
Adult worms

Fig 2.4

Life cycle of *N. brasiliensis* in rat

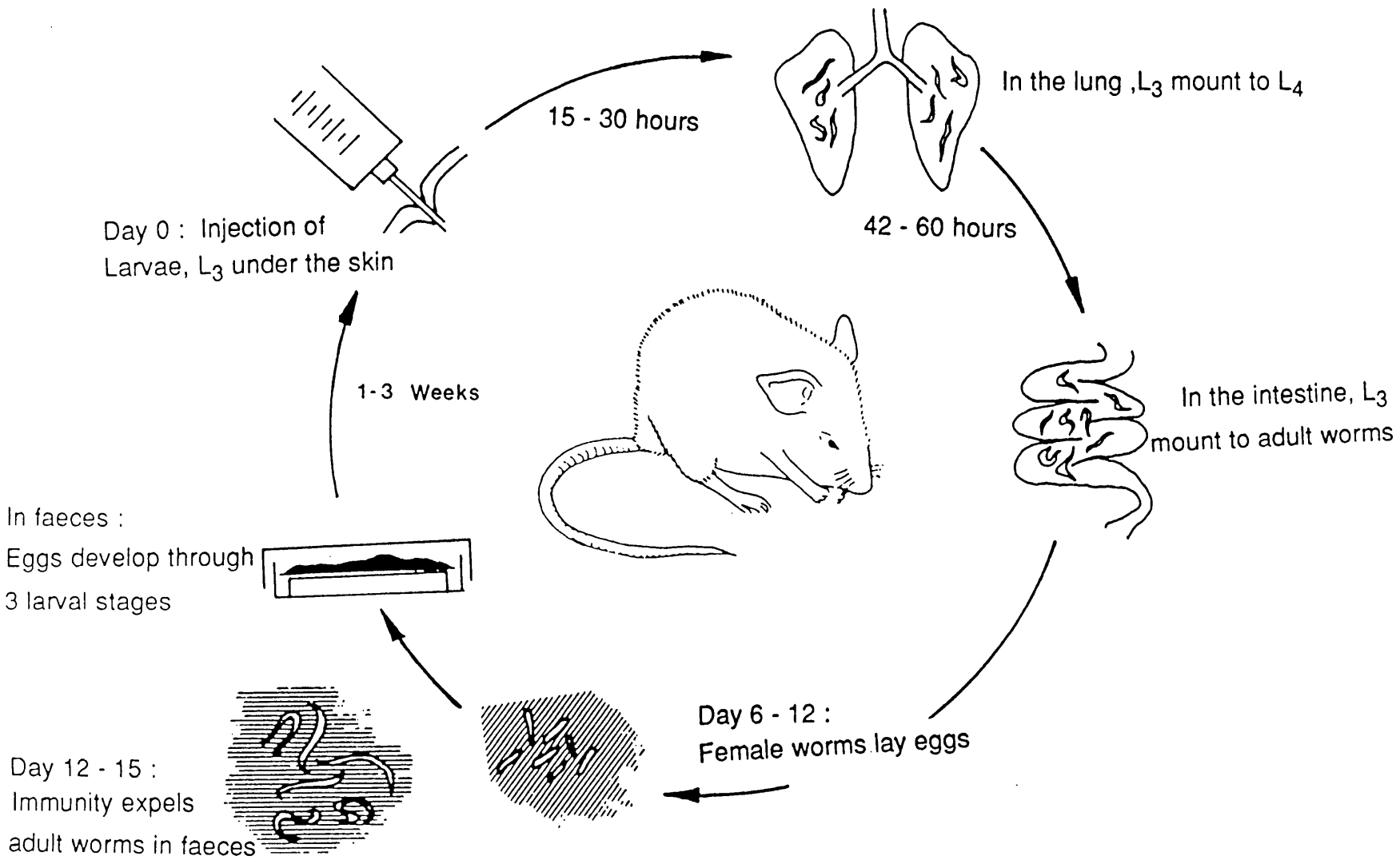


Fig 2.5

CHAPTER THREE

MAST CELL HETEROGENEITY ACROSS DIFFERENT SPECIES

3.1 Introduction

The pathophysiological stimulus for the release of histamine from the mast cell is provided by the combination of specific antigen with reaginic antibody fixed to the cell surface [44,45]. In addition, secretion may also be induced by a variety of pharmacological agents which act independently of the immunological mechanism [297]. One group of such agents, consisting of polybasic compounds and neuropeptides, has been extensively employed in the functional study of mast cell heterogeneity [279-281,298].

Indeed, apart from the heterogeneous effects observed in isolated mast cells in vitro, these agents can also produce the same phenomenon in vivo [299,300]. Compound 48/80 for example, when given parenterally, produces a severe or fatal anaphylactoid reaction in the rat, cat and dog but has a limited or negligible effect in the rabbit, mouse, guinea pig, hamster, man and monkey [299,300]. The systemic responses in the former species are accompanied by a marked degranulation of mast cells and a depletion of histamine in a variety of connective tissues.

The introduction of the drug disodium cromoglycate (DSCG) has undoubtedly provided a major advance in the treatment and prophylaxis of asthma and other allergic conditions. Although the mode of action of the drug may be more complex than initially thought, the clinical utility is generally ascribed at least in part to its ability to inhibit mediator release from tissue mast cells. However, as has already been described in section 1.6.3, the compound exhibits a high degree of specificity in its action. This therefore highlights the difficulty in extraplotating results from one animal system to another in the screening of novel compounds that may modulate the activity of the mast cell.

With this in mind, it is the aim of the present study to investigate the heterogeneous behaviour of mast cells from a number of different species, employing a variety of polycationic compounds ranging from the 'classical mast cell degranulating agent' compound 48/80, to polyamino acids and neuropeptides. At the same time, this phenomenon is also examined by looking at the effects of two anti-allergic agents, DSCG and its more active congener, nedocromil sodium.

3.2 Methods

All methods used in this study were as described in chapter two.

3.3 Results

3.3.1 Histamine release by compound 48/80 and polymyxin

The synthetic polyamine compound 48/80 (fig 3.1) and the antibiotic polymyxin (fig 3.2) both induced a pronounced secretion of histamine from rat peritoneal and pleural mast cells. The hamster cells were significantly less responsive especially at low concentrations of the secretagogues. Peritoneal mast cells of the mouse only released histamine at high drug concentrations while those cultured from bone marrow (BMCMC) showed negligible reactivity. Moreover, enzymically dispersed mast cells from the rat intestinal mucosa, guinea pig lung and mesentery, pig lung, human lung and isolated human basophils were only weakly responsive or were essentially refractory to the action of the two agents.

3.3.2 Histamine release by substance P and $SP_{1-4}C_{12}$

The neuropeptide substance P (fig 3.3) and $SP_{1-4}C_{12}$ (fig 3.4), a compound synthesized by forming a peptide bond between Arg-Pro-Lys-Pro, the N-terminal sequence of substance P, and dodecylamine [301], were potent releasers of histamine from rat peritoneal and pleural mast cells. In rat peritoneal mast cells, $SP_{1-4}C_{12}$ was about 200 times more active than substance P; the EC_{50} values (concentrations required to elicit 50 % histamine release) were 81.1 nM for $SP_{1-4}C_{12}$ and 15 μM for substance

P. Peritoneal mast cells from the hamster and mouse and those enzymically isolated from the rat intestinal mucosa were only weakly responsive while BMCMC and mast cells from the guinea pig lung and mesentery, pig lung, human lung and isolated human basophils were unreactive to the histamine releasing action of the two agents.

3.3.3 Histamine release by polyamino acids

Rat peritoneal and pleural mast cells responded strongly to the polyamino acids polylysine (fig 3.5) and polyarginine (fig 3.6). In contrast to the polybasic compounds discussed in the previous two sections, comparable activity especially to polyarginine was also observed in the hamster cells. Mast cells from the mouse peritoneum, and to a lesser extent rat intestinal mast cells and BMCMC released a moderate but consistent percentage of their total histamine in response to the two agents with polyarginine again the more active of the two. Human basophils also responded to the polyamino acids with polyarginine releasing nearly 50 % of the total histamine at a concentration of 100 μg/ml. The other mast cell types, namely those derived from the guinea pig lung and mesentery, pig lung and human lung were again essentially unresponsive.

3.3.4 Histamine release by polybasic secretagogues: effect of calcium

With the exception of substance P, maximal histamine release from rat peritoneal mast cells induced by the other five basic secretagogues was greatest in the presence of external calcium (1 mM) (figs 3.7-3.9). A considerable release of the amine was still observed in the absence of the cation and following brief treatment (5 min) with the calcium chelator EDTA (0.1 mM). Moreover, at low drug concentrations for all six polybasic secretagogues, histamine release was normally higher in the absence than in the presence of calcium and was potentiated by the brief treatment with EDTA (figs 3.7-3.9).

3.3.5 Inhibition by DSCG and nedocromil sodium

The prototype anti-allergic drug DSCG was a potent inhibitor of immunologically induced histamine release from rat peritoneal and pleural mast cells (fig 3.10). The drug

was less active against peritoneal mast cells from the hamster and completely ineffective against these cells from the mouse. The chromone was only weakly active against human lung mast cells and virtually ineffective against mast cells from the rat intestine, guinea pig lung and mesentery, pig lung, and human basophils. Nedocromil sodium, a more recently developed congener of DSCG, showed a similar pattern of differential responses between the different species (fig 3.11), in addition to being at least one order of magnitude more potent than DSCG in inhibiting immunologically induced histamine release from human lung mast cells.

3.4 Discussion

The present study has shown that isolated mast cells from different species are functionally heterogeneous in their responses to various polycationic compounds and to the inhibitory effects of anti-allergic chromones.

In general, the 'classical mast cell degranulating agent' compound 48/80 and the antibiotic polymyxin followed a similar pattern of activity in vivo and in vitro. Upon parenteral adminstration, the two agents produced severe or fatal anaphylactoid reactions in the rat, cat and dog but had limited or negligible effects in a variety of other species including the rabbit, mouse, guinea pig, hamster, man and monkey [299,300,302]. In the present study, both compound 48/80 and polymyxin were potent releasers of histamine from rat peritoneal and pleural mast cells. Peritoneal mast cells of the hamster, and particularly of the mouse, were significantly less reactive. BMCMC and enzymically dispersed mast cells from the rat intestinal mucosa, guinea pig lung and mesentery, pig lung, human lung and isolated human basophils were only very weakly responsive or were essentially refractory to the two agents.

The neuropeptide substance P also exhibited a similar spectrum of specificity. In accordance with compound 48/80 and polymyxin, the undecapeptide produced a pronounced secretion of histamine from rat peritoneal and pleural mast cells, became much less active against peritoneal mast cells of the hamster and mouse and was virtually ineffective against the other mast cell types studied. However, in agreement with the results of Shanahan et al [303], substance P induced a weak but consistent

release of histamine from enzymically dispersed rat intestinal mast cells.

The ability of substance P to release histamine from mast cells may bear more important significance. There is currently considerable interest in the potential interaction between mast cells and peptidergic nerves in the pathogenesis of allergy and inflammation. Tissue mast cells are frequently found in intimate contact with sensory nerves and in some cases the apparent existence of discrete neuroeffector junctions has been demonstrated [304-306]. In addition, substance P at subnanomolar concentrations released histamine from human skin after intradermal injection [307] and a number of sensory neuropeptides including substance P have been shown to release histamine from isolated rat serosal and human skin mast cells [308-310]. These observations have been incorporated into a generalized axon-reflex mechanism for neurogenic inflammation [310,311]. Activation of polymodal nociceptors generates impulses to pass orthodromically along primary afferent nerve fibres to the dorsal horn of the spinal cord and antidromically to terminal arborizations of the same fibre [310,311]. Such antidromic impulses may lead to the release of sensory neuropeptides, such as substance P, which in turn stimulate mast cells to release histamine and other chemical mediators, thereby amplifying the inflammatory response.

The validity of the above hypothesis as applied to man has been tested recently in a number of separate laboratories. Pearce et al [280,281] investigated the effects of a variety of basic, sensory neuropeptides on different mastocytes and found that these agents, which included calcitonin-gene-related peptide (CGRP), peptide histidine methionine (PHM), somatostatin (Som), substance P (SP) and vasoactive intestinal peptide (VIP) were strikingly similar in their tissue and species selectivity as compared to the polyamine compound 48/80. Indeed, in accordance to compound 48/80, these neuropeptides were most active against rat serosal mast cells but had very little or no effect against human basophils and enzymically isolated mast cells from lung parenchyma and colonic mucosa [280,281]. In another study, Lowman et al [309] found that CGRP, SP, Som and VIP all produced weak histamine release from enzymically isolated human skin mast cells. Under these circumstances, it is therefore difficult to sustain a generalized model of neurogenic inflammation involving mast cell-peptide interactions in man, except possibly in skin. Indeed, it was suggested [280,281] that

basic neuropeptides and other polyamines may act through a common receptor or binding site that is non-specific, of uncertain significance and largely confined to particular murine mast cells. These suggestions do not, of course, detract from the potential importance of direct inflammatory effects of sensory neuropeptides nor do they preclude the existence of alternative form of communication, in either direction, between mast cells and nerves.

The ability of basic compounds to release histamine from certain mast cell types has been firmly established in the present study. However, the precise mechanism behind this release is still the subject of great uncertainty. Studies by Jasani et al [312] and Stanworth et al [313,314] with a variety of peptides derived from adrenocorticotrophic hormone and other synthetic peptides comprising sequences in the $C_\varepsilon 4$ domain of the human IgE molecule have revealed certain structural requirements for histamine-releasing peptides; these were found to be basic amino acid residues such as lysine and arginine at the N-terminus and hydrophobic amino acid residues at the C-terminus. Moreover, Fewtrell et al [315], in an extensive structure-activity study of substance P-related peptides on rat peritoneal mast cells, have shown that the N-terminal basic amino acid residues arginine and lysine at positions 1 and 3, respectively, were essential for histamine releasing activity whereas progressive shortening of the C-terminal sequence in the series SP_{1-9}, SP_{1-8}, SP_{1-7} and SP_{1-6} produced a corresponding reduction in the ability of the peptides to release histamine.

Repke and Bienert [316], in an attempt to clarify this situation further, synthesized a number of substance P analogues with modifications both in the N-terminus and C-terminus of the undecapeptide and then studied their effects on both rat and hamster peritoneal mast cells. They found that blocking the basic amino acid residues arginine and lysine at the N-terminus with large chemical groups completely abrogated the histamine releasing activity of substance P. In contrast, analogues that contained modifications which resulted in an increased hydrophobicity of the C-terminus enhanced the histamine releasing potency and vice versa. Modifications were not dependent on hydrophobic amino acid residues since the compound Arg-Pro-Lys-Pro-$(CH_2)_{11}CH_3$ ($SP_{1-4}C_{12}$), synthesized by forming a peptide bond between the N-terminal sequence of substance P and the 12-carbon aliphatic chain of dodecylamine, was one

of the most active analogues tested. Indeed, in the present study, $SP_{1-4}C_{12}$ was about 200 times more active than substance P on rat peritoneal mast cells and was also generally more active on tissue mast cells of the same species [chapter 4]. With this in mind, Repke et al [301,316] proposed the following structural requirements which are necessary for histamine release by basic molecules: 1) residues within the molecule which bear a positive charge such as the arginine and lysine residues of substance P or the amino groups of compound 48/80, and 2) the presence of a hydrophobic structure such as the C-terminal sequence of substance P, the aliphatic chain in $SP_{1-4}C_{12}$ or the benzene rings of compound 48/80. Moreover, it is necessary for the basic groups to be coupled to the hydrophobic part of the molecule in order to activate histamine release since neither the N-terminal tetrapeptide SP_{1-4} nor the C-terminal heptapeptide SP_{5-11} of substance P expressed any significant activity when compared to the full undecapeptide [316]. The authors went further to propose that histamine release induced by polybasic molecules is activated by insertion of a hydrophobic moiety into the mast cell membrane which then permits the positively charged species to interact with a receptor or binding site which triggers the secretory process [301].

The above hypothesis is probably only relevant to certain mast cell types, notably those derived from the serosal cavities of the rat, since in the present study, isolated mast cells from man, guinea pig and pig were essentially refractory to the histamine releasing action of these polybasic agents. Indeed, the relevance of this theory even to rat serosal mast cells is questioned by the results obtained with the polyamino acids. Both polylysine and polyarginine were potent releasers of histamine from rat peritoneal and pleural mast cells. Comparable activity especially to polyarginine was also observed in hamster peritoneal mast cells. Mast cells from the mouse peritoneum, and to a lesser extent rat intestinal mast cells and BMCMC released a moderate but consistent percentage of their total histamine in response to the two agents. In stark contrast to the other basic compounds, human basophils also responded to the polyamino acids, with polyarginine again showing particularly high activity. The other mast cell types, namely those derived from the guinea pig lung and mesentery, pig lung and human lung were essentially unresponsive. These data therefore raise doubts concerning the requirement for a hydrophobic region to initiate the secretory process.

The unusual effect of the polyamino acids on human basophils requires some further comment. This phenomenon was probably first reported by Foreman and Lichtenstein [317] who demonstrated histamine release from human basophils induced by polyarginine, polylysine and polyornithine. Moreover, the release was dependent on the chain length of the polymer and the constituent amino acid. They proposed that the effect of chain length on release potency might simply be related to the importance of the number of amino groups or positive charges while the activity of a certain polymer might be linked to the ability of the constituent amino acid to donate a proton. The resultant positively charged polymer might then interact with negatively charged moieties in the cell membrane such as sialic acid residues.

Finally, it is worth mentioning that apart from the similarity in their species selectively, these basic agents also shared many common patterns in their fundamental characteristics of histamine release [281]. One of these characteristics, as demonstrated in the present study, is the ability to initiate secretion in the absence of extracellular calcium, thus indicating their ability to mobilize intracellular stores of this cation.

The widespread involvement of the mast cell in a diversity of allergic disorders has naturally led to attempts to develop drugs that suppress their function. Such drugs would represent an obvious therapeutic development since agents that prevent the release of inflammatory mediators should provide a means of controlling the earliest stages of the allergic response. One such compound, disodium cromoglycate (DSCG) has been widely used in the treatment of human bronchial asthma, and although its basic mechanism is still the subject of controversy, the clinical utility of the drug has been generally attributed, at least in part, to its ability to inhibit mediator release from tissue mast cells [283,302,318,319].

The compound, however, shows a very high degree of species and tissue selectivity. In human asthmatics, DSCG inhibits allergen induced bronchoconstriction when used prophylactically [318,319]. In the rat, the compound effectively suppresses cutaneous, peritoneal and pulmonary anaphylactic reactions in vivo [319-321] but has no effect on cutaneous or respiratory anaphylaxis in the guinea pig [319].

The present study, and in agreement with other published reports [221,257,279,302], has demonstrated that the specificity of DSCG also extends to isolated mastocytes in vitro. Indeed, DSCG was a potent inhibitor of immunologically induced histamine release from rat serosal mast cells. The drug was, however, less active against peritoneal mast cells from the hamster and completely ineffective against these cells from the mouse. The chromone, for all its clinical utility, was only weakly active against human lung mast cells while histamine release from rat intestinal mast cells, and in addition those from the guinea pig lung and mesentery, pig lung, and human basophils was unaffected.

Nedocromil sodium is a recently introduced anti-asthmatic agent with structural similarities to DSCG and is thought to exert its effects via a similar mechanism [322]. Nedocromil sodium also showed a high degree of specificity against isolated mastocytes from different sources. Indeed, in the present study, its profile of activity was virtually identical to that observed for DSCG. Perhaps one major difference between the two chromones was the higher activity of nedocromil sodium on human lung mast cells.

The effect of the chromones on human pulmonary mast cells merits some further comment. Despite their clinical utility, the two drugs are only weakly to moderately active on mast cells from the lung parenchyma. However, there are now extensive data to show that the chromones are much more active against mast cells obtained by bronchoalveolar lavage (BAL) [283-285,323]. In this respect, it can be argued that different pulmonary mast cell subpopulations may play differing roles in the pathogenesis of asthma. The BAL cells lie presumably within or immediately adjacent to the mucosal surface of the airways and may then be of major importance in modulating the initial phases of the allergic response. The cells would come into direct contact with inhaled antigens and release their mediators locally onto the airway surface. More deeply situated mast cells and other inflammatory cells may become progressively involved in the development of the chronic disease, with damage to the mucosal surface and respiratory epithelium allowing an increased penetration of inhaled antigen.

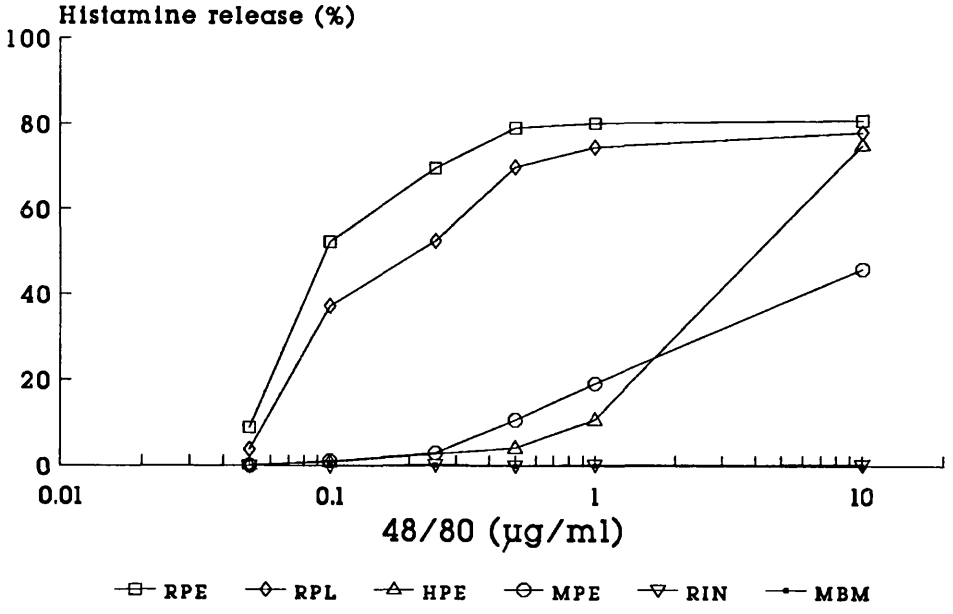
In total, the present study clearly emphasise the danger in interspecies

extrapolation in the assessment of potential mast cell liberators and anti-allergic agents.

Abbreviations for figures 3.1-3.11

CMF	- In calcium-free-Tyrode's
ET	- In calcium-free-Tyrode's containing EDTA
FHT	- In Full HEPES-Tyrode's
GPM	- Guinea pig mesenteric mast cells
GPL	- Guinea pig lung mast cells
HB	- Human basophils
HL	- Human lung mast cells
HPE	- Hamster peritoneal mast cells
MBM	- Mouse bone marrow cultured mast cells
MPE	- Mouse peritoneal mast cells
PL	- Pig lung mast cells
RIN	- Rat intestinal mast cells
RPE	- Rat peritoneal mast cells
RPL	- Rat pleural mast cells

Fig 3.1 Histamine release induced by compound 48/80 from isolated mast cells from various sources. Results are based on at least four experiments and error bars are omitted for clarity but did not exceed 7 %.



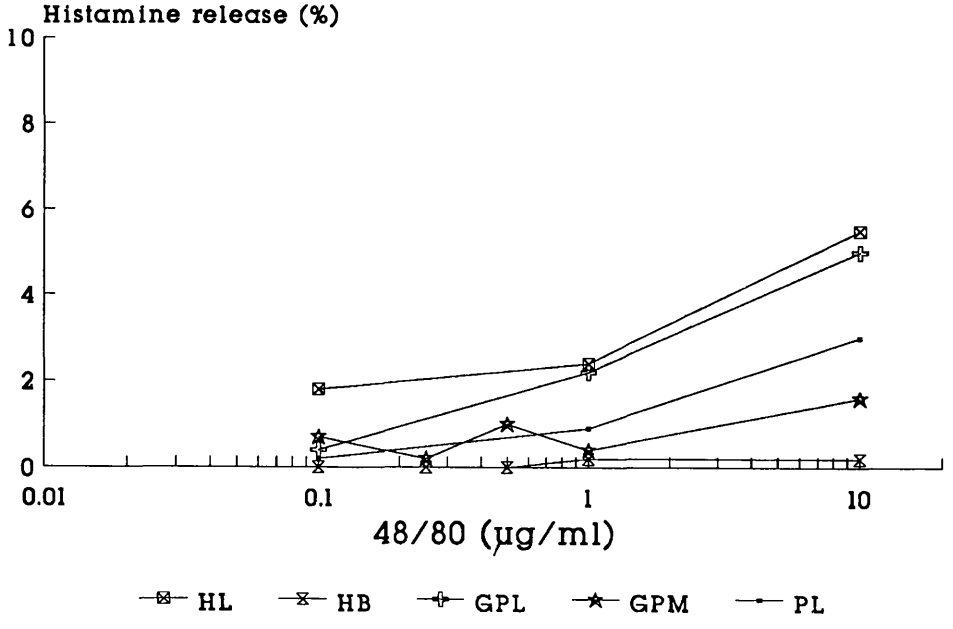
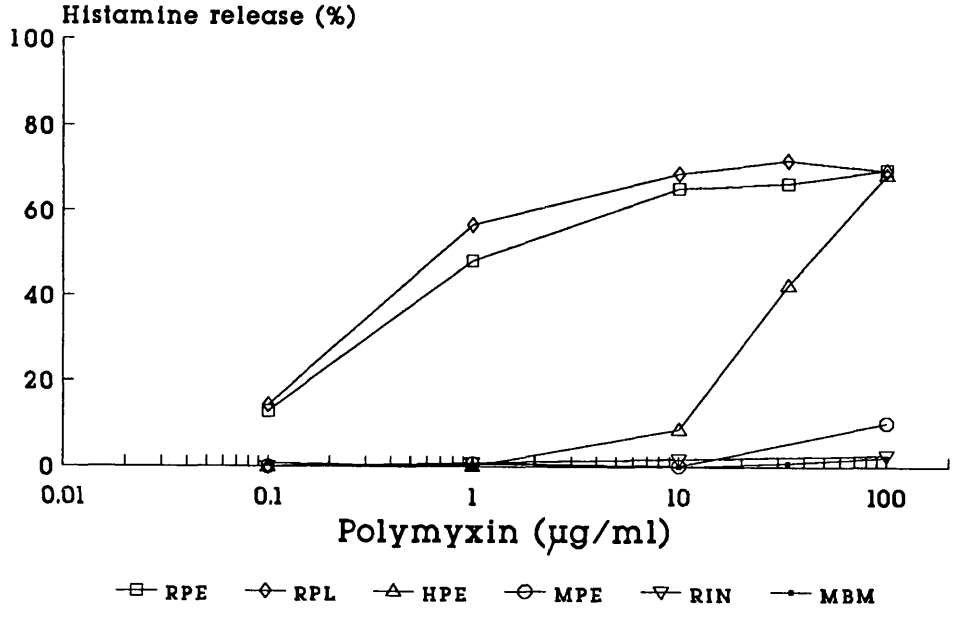


Fig 3.2 Histamine release induced by polymyxin from isolated mast cells from various sources. Results are based on at least four experiments and error bars are omitted for clarity but did not exceed 9 %.



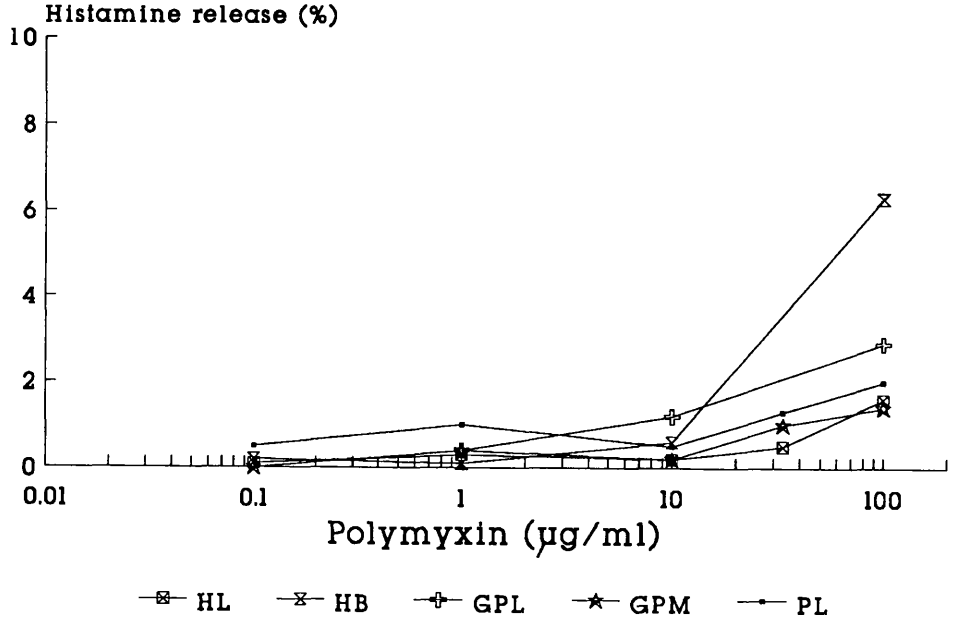
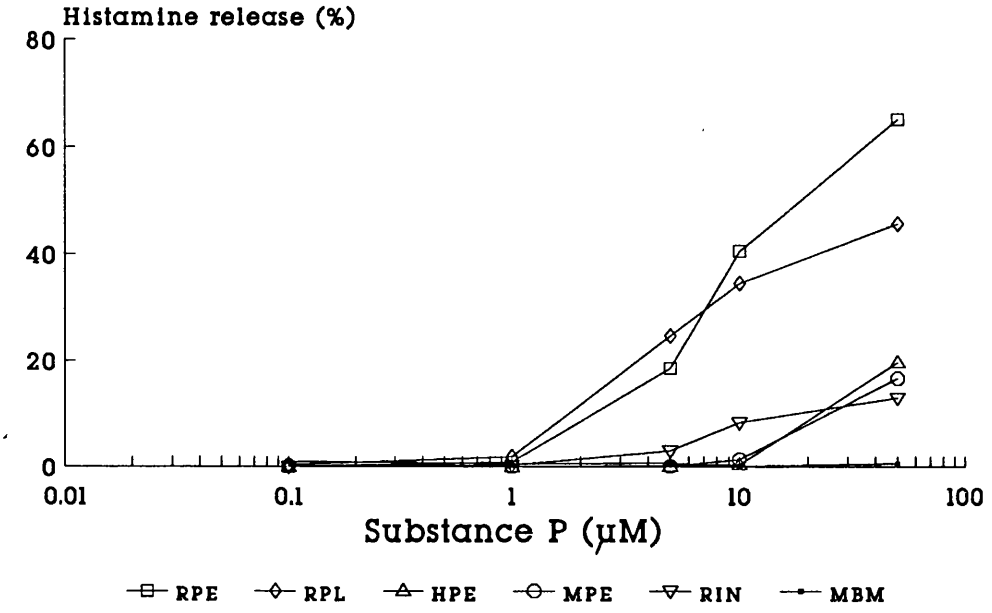


Fig 3.3 Histamine release induced by substance P from isolated mast cells from various sources. Results are based on at least four experiments and error bars are omitted for clarity but did not exceed 4 %.



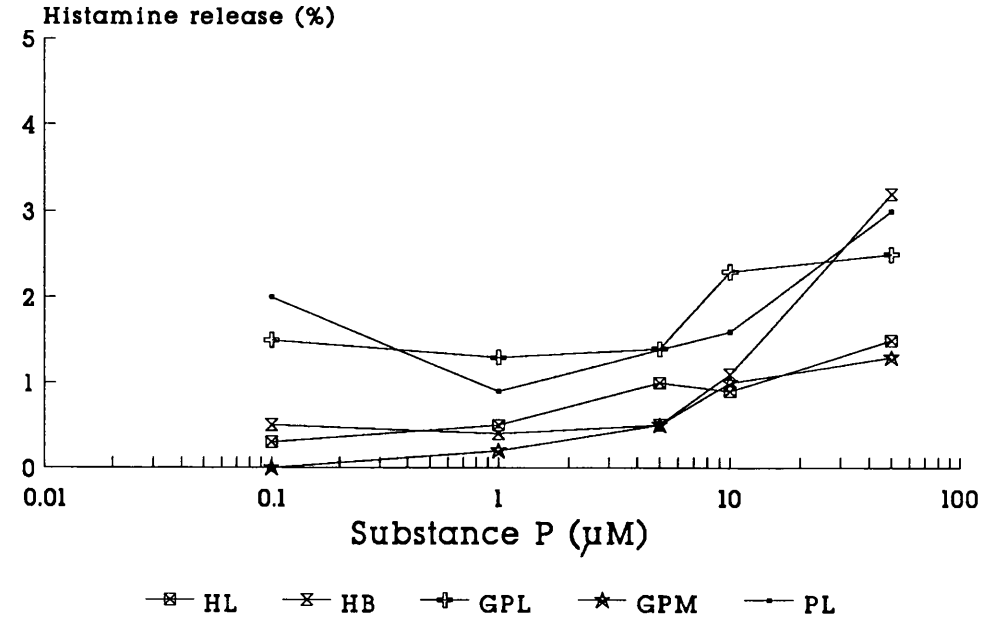
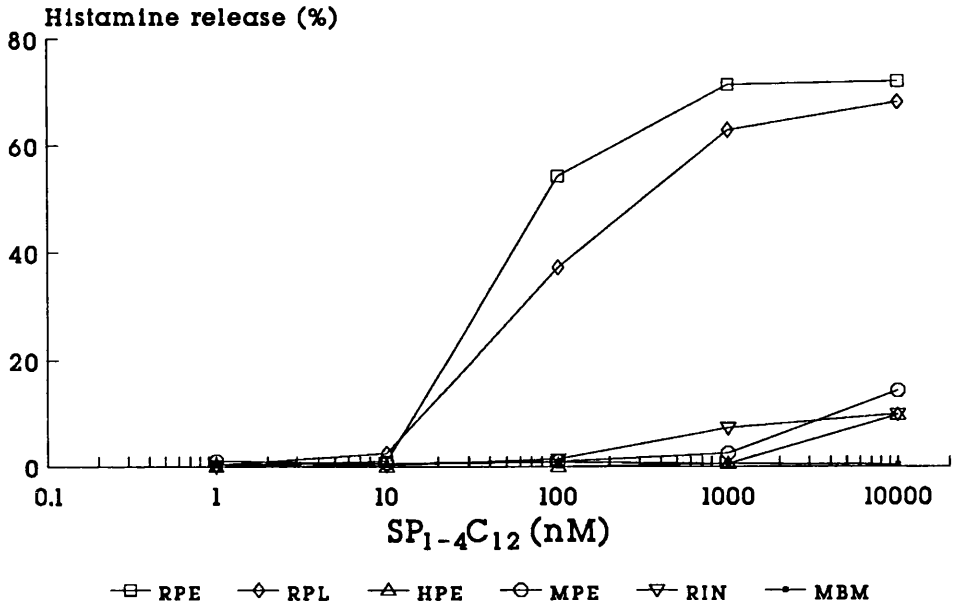


Fig 3.4 Histamine release induced by $SP_{1-4}C_{12}$ from isolated mast cells from various sources. Results are based on at least four experiments and error bars are omitted for clarity but did not exceed 10 %.



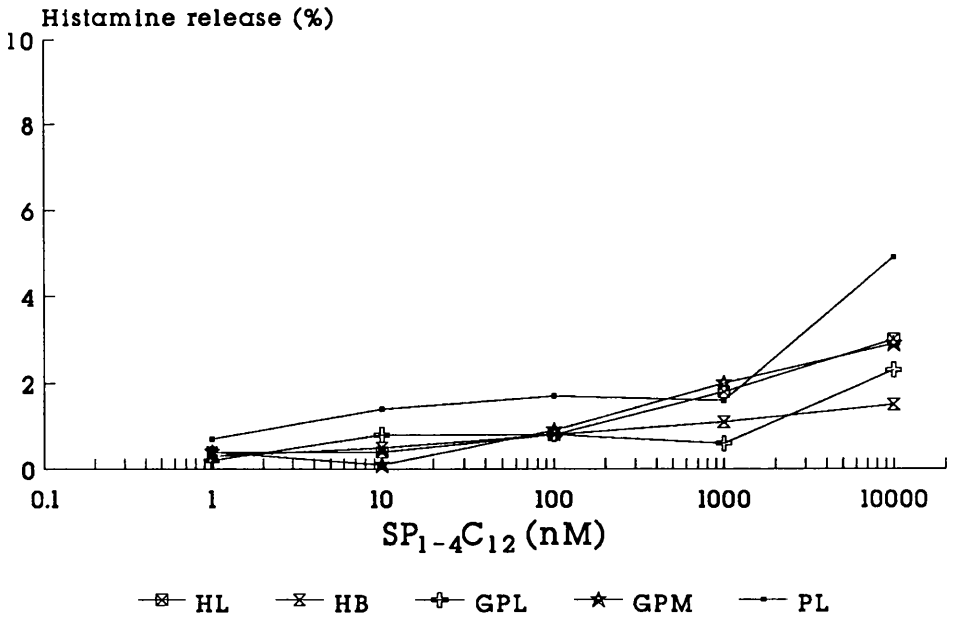
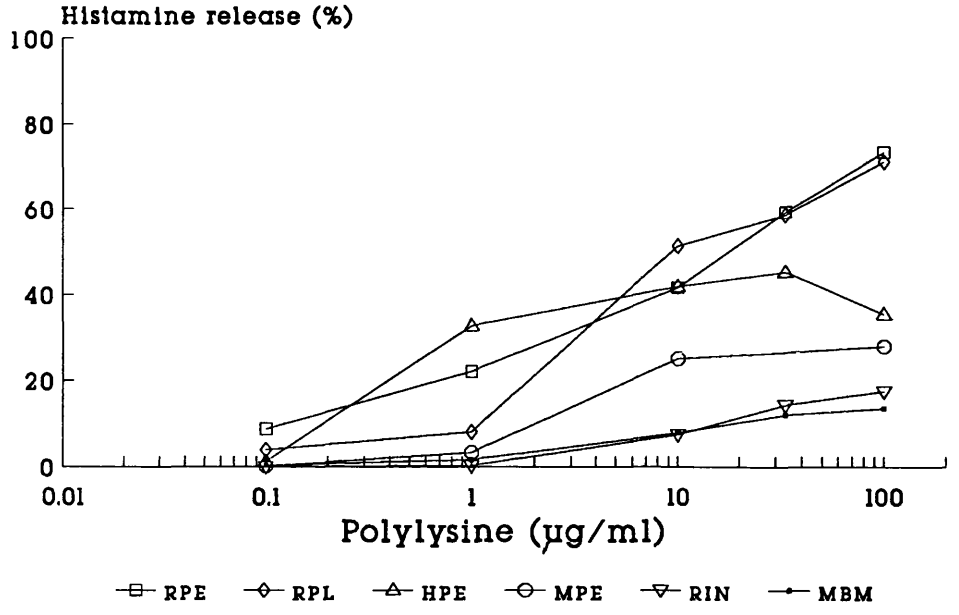


Fig 3.5 Histamine release induced by polylysine from isolated mast cells from various sources. Results are based on at least four experiments and error bars are omitted for clarity but did not exceed 7 %.



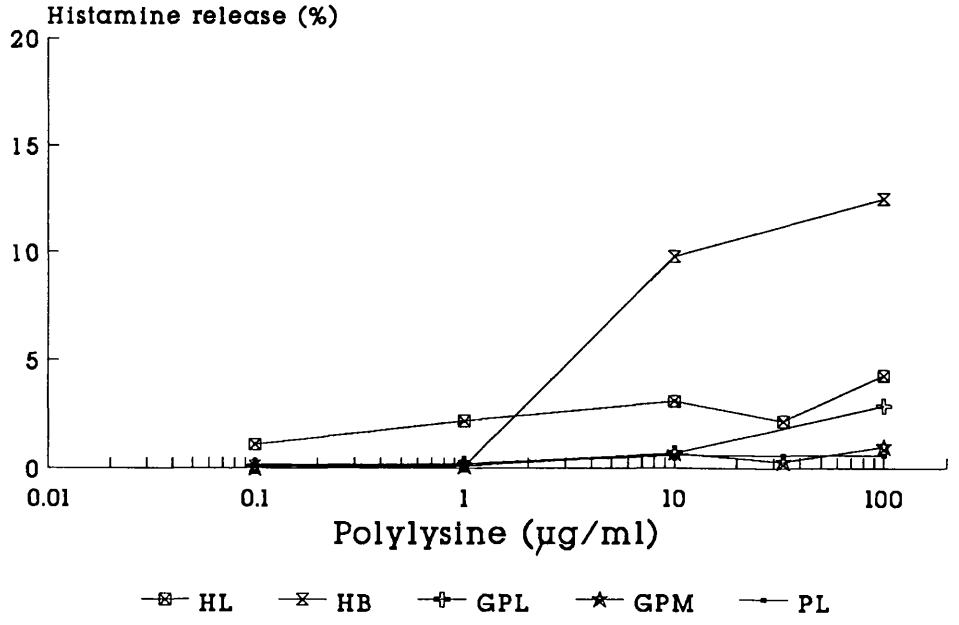
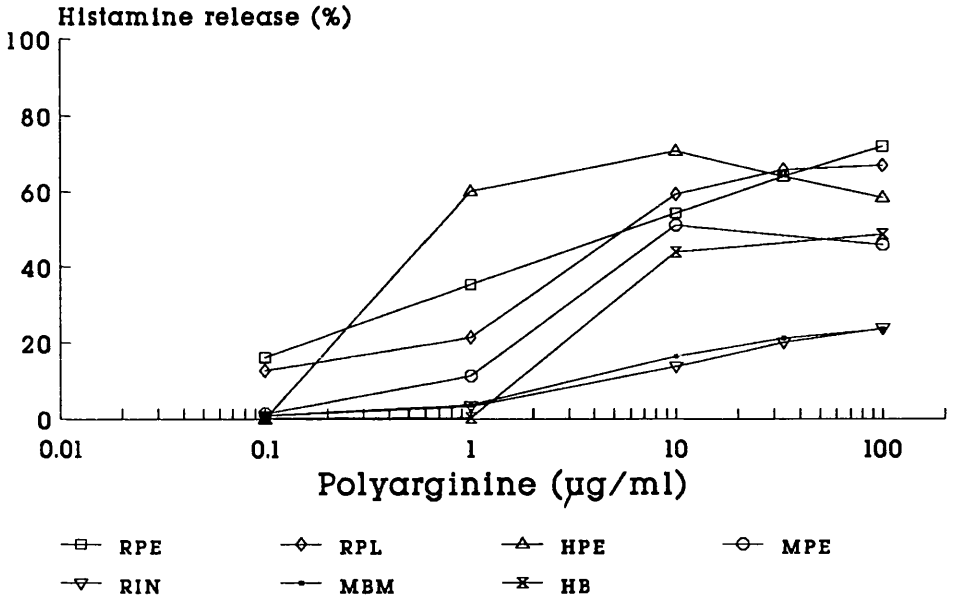


Fig 3.6 Histamine release induced by polyarginine from isolated mast cells from various sources. Results are based on at least four experiments and error bars are omitted for clarity but did not exceed 6 %.



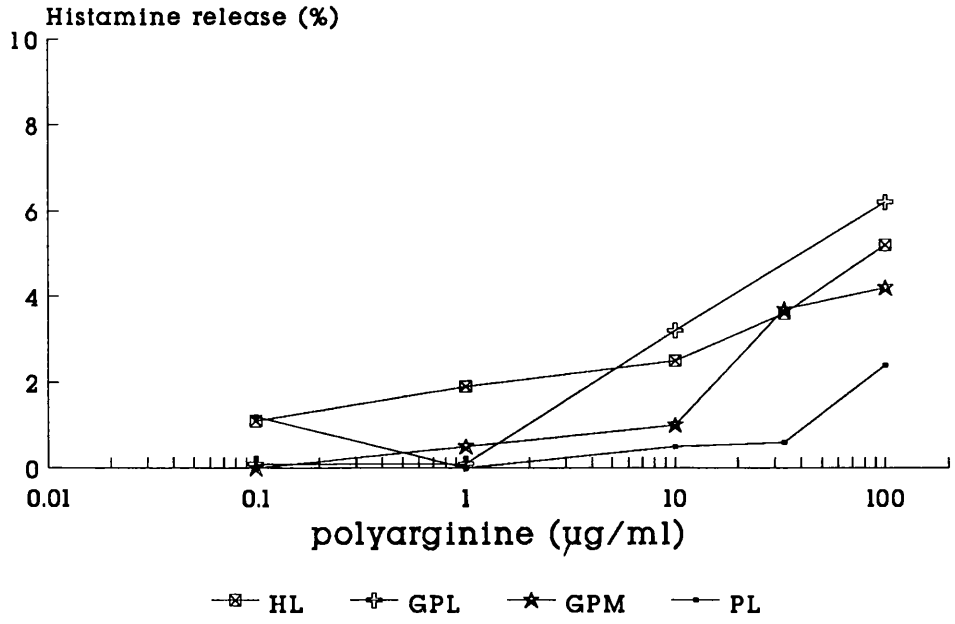
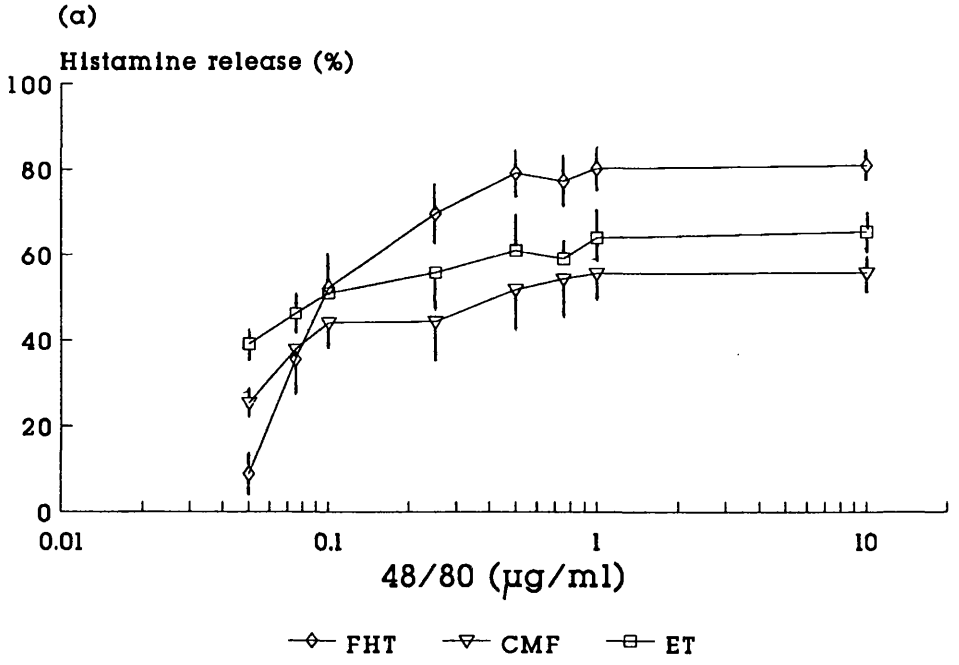


Fig 3.7 Histamine release induced by (a) compound 48/80 and (b) polymyxin in Full (FHT), calcium-free (CMF) and EDTA (ET) Tyrode's solution from rat peritoneal mast cells.



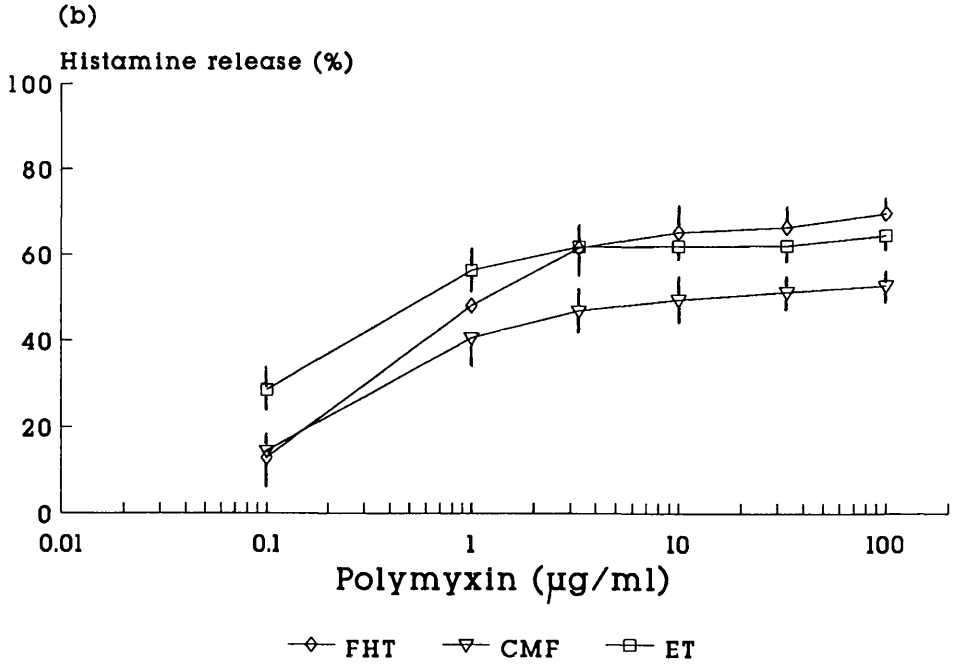
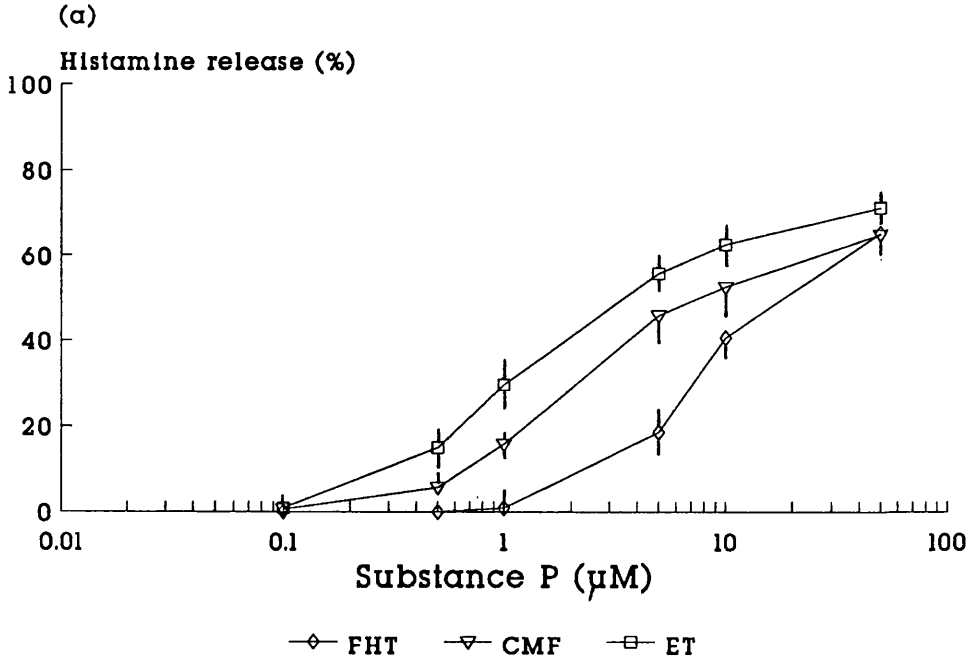


Fig 3.8 Histamine release induced by (a) substance P and (b) $SP_{1-4}C_{12}$ in Full (FHT), calcium-free (CMF) and EDTA (ET) Tyrode's solution from rat peritoneal mast cells.



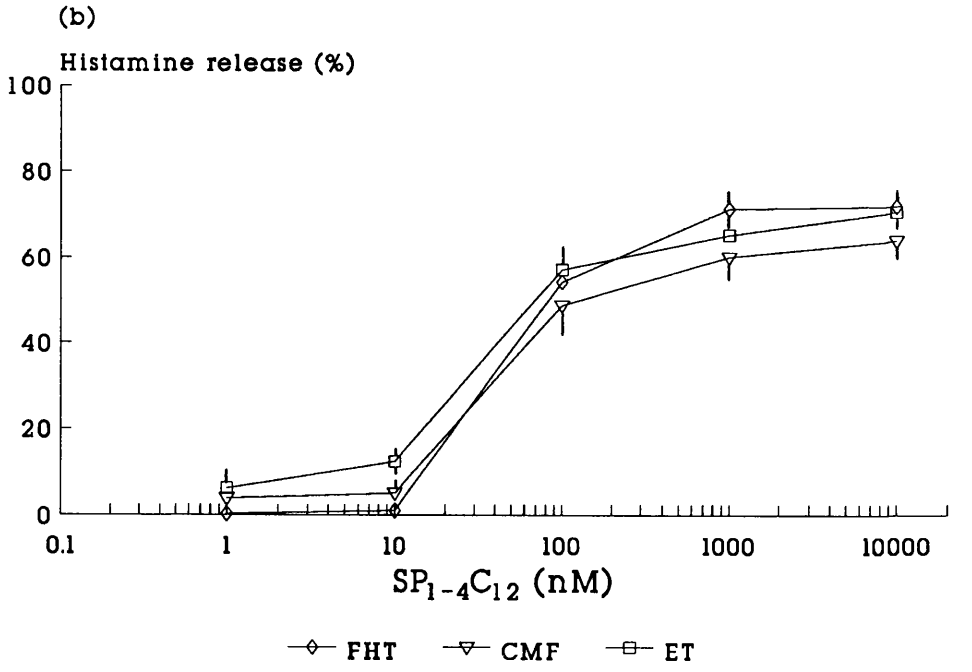
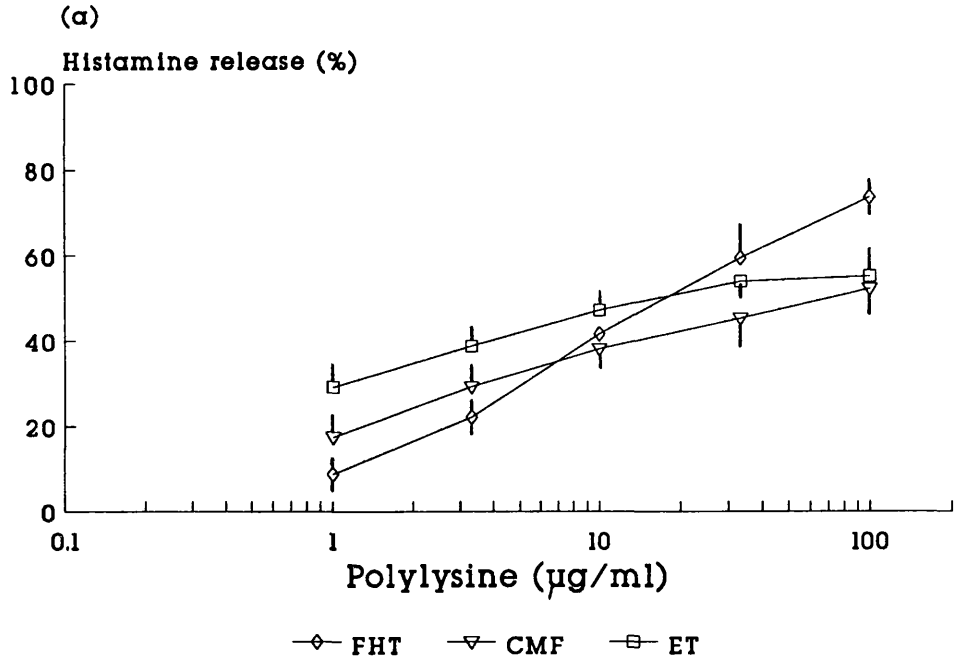


Fig 3.9 Histamine release induced by (a) polylysine and (b) polyarginine in Full (FHT), calcium-free (CMF) and EDTA (ET) Tyrode's solution from rat peritoneal mast cells.



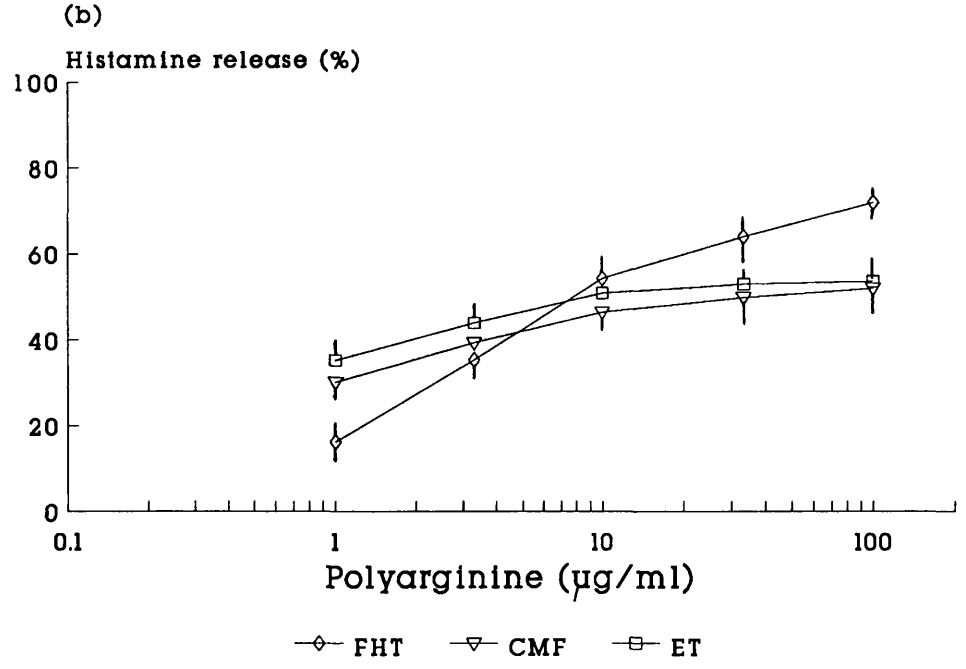
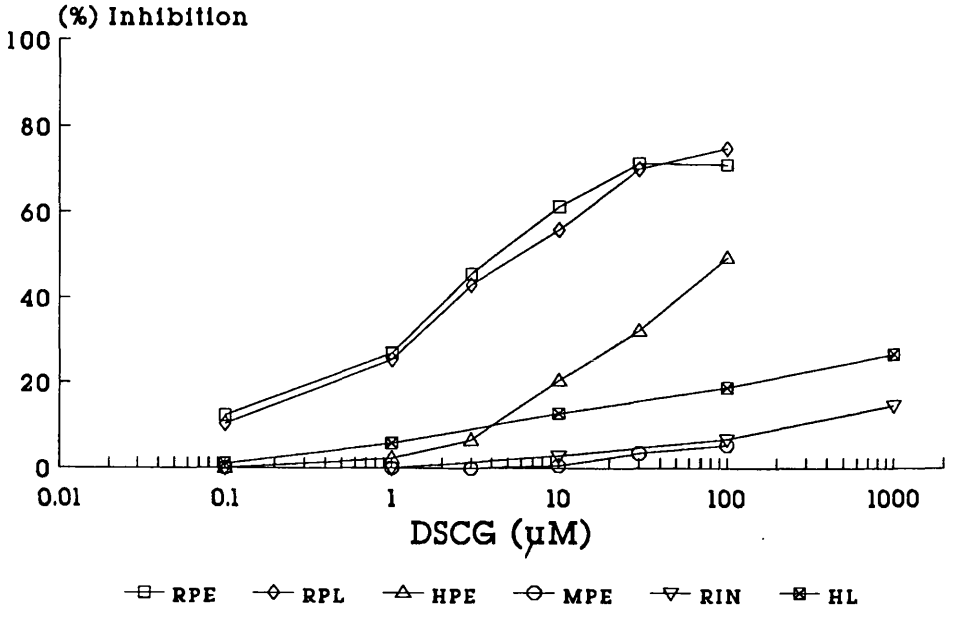


Fig 3.10 Inhibition by disodium cromoglycate (DSCG) of anaphylactic histamine release from isolated mast cells from various sources. Results are based on at least four experiments and error bars are omitted for clarity but did not exceed 10 %.



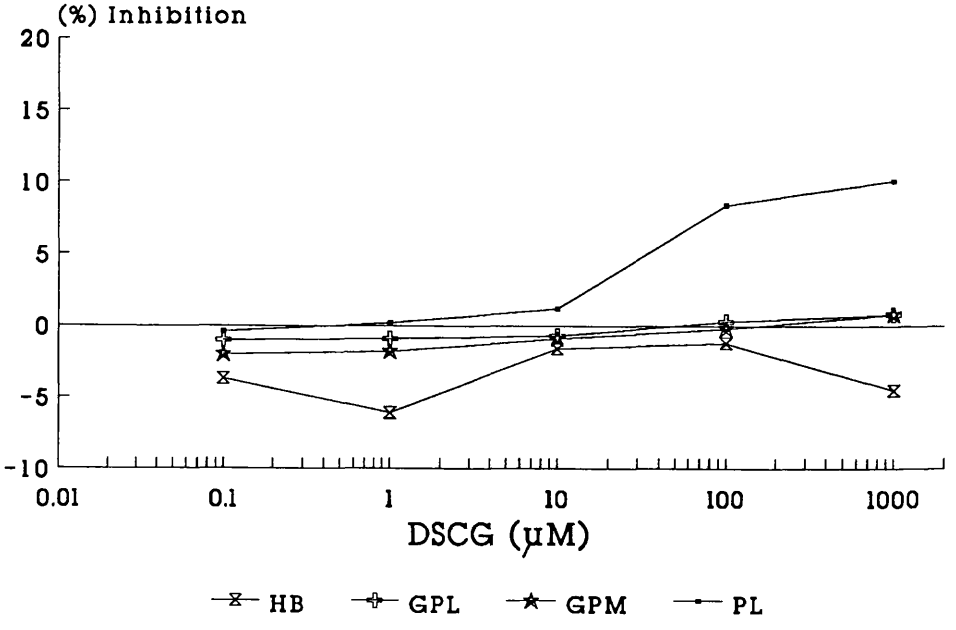
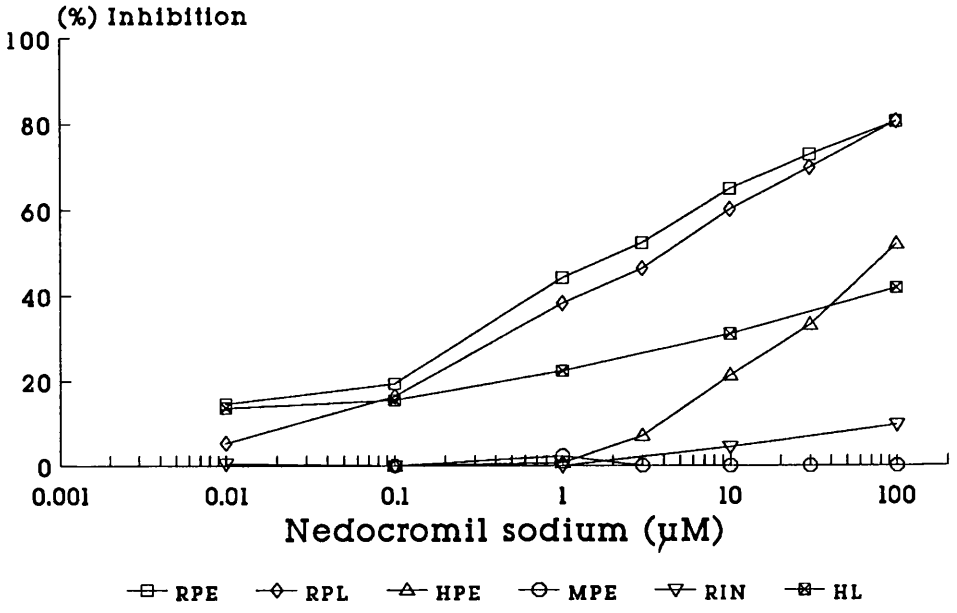
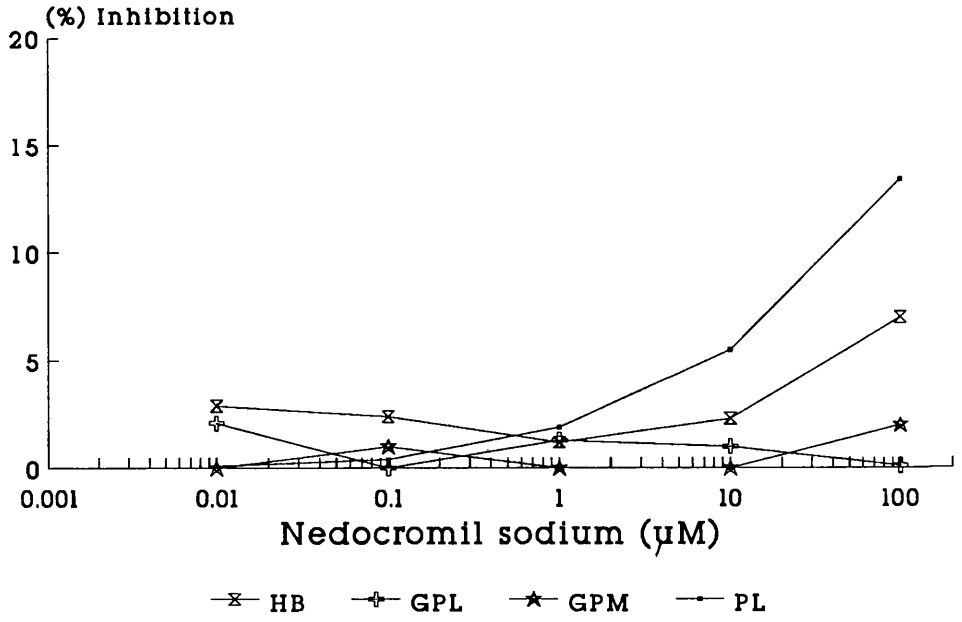


Fig 3.11 Inhibition by nedocromil sodium of anaphylactic histamine release from isolated mast cells from various sources. Results are based on at least four experiments and error bars are omitted for clarity but did not exceed 10 %.





CHAPTER FOUR

MAST CELL HETEROGENEITY IN THE RAT: EFFECTS OF HISTAMINE LIBERATORS

4.1 Introduction

The initial observation by Maximow [254] and the subsequent series of classic experiments by Enerbäck [255,324-326] have provided unequivocal evidence concerning the existence of two distinct types of mast cell in the gastrointestinal tract of the rat. The mast cells located in the lower layer of the intestinal wall resemble those found in other connective tissues and in the serosal cavities. The cells in the mucosa, however, show very different properties. They are generally smaller in size and more variable in shape than the connective tissue cells, have a lower content of histamine and 5-hydroxytryptamine and possess fewer granules. Moreover, they also exhibit numerous biochemical, functional and histochemical characteristics which are distinct from their connective tissue counterparts (table 1.1).

The terms mucosal mast cell(s) (MMC) and connective tissue mast cell(s) (CTMC) have been introduced to describe these two distinct populations in the gastrointestinal tract of the rat. Moreover, MMC but not CTMC have the unique property of proliferating in response to intestinal helminth infection [327,328]. This MMC response has been studied in detail in rats infected with the nematode Nippostrongylus brasiliensis [328,329]. After skin penetration, infective larvae migrate to the intestine via the lungs, trachea and esophagus, molt to adult worms and start to lay eggs, which are passed in the faeces. The worms are predominantly localized in the proximal part of the jejunum [328]. At 8-10 days after the infection, MMC are virtually absent from the lamina propria and it has been proposed that this effect might be due to the presence of a non-specific mast cell degranulator produced by the worms [255,328]. The mast cell degranulation is followed by a proliferation of MMC, reaching a maximum level of about 5 times the normal number on day 12-14 after the infection. This also coincides with the expulsion of worms from the gut [328].

This unique response to helminth infection has greatly facilitated the isolation of these intestinal MMC for functional studies. In a series of pioneering experiments, Befus, Pearce and their co-workers [65,330,331] investigated the properties of enzymically isolated mast cells from the lamina propria of the small intestine and compared them to the more conventional peritoneal mast cells. With this in mind, it is the aim of the present study to extend the above investigation even further by also including mast cells from the lung and mesentery.

4.2 Methods

All methods used in this study were as described in Chapter 2.

4.3 Results

4.3.1 Basic characteristics of rat mast cells

The enzyme collagenase was found to be effective in the dispersion of rat mesenteric, lung and intestinal tissues into free cell suspensions. Mast cells comprised 5.3±0.6 (peritoneum), 4.2±0.7 (mesentery), 3.5±0.6 (lung) and 12.7±1.9 % (intestine) of the total nucleated cells (n=6-8) (table 4.1). The histamine contents per mast cell were 24.3±2.3, 7.6±0.8, 3.8±0.3 and 1.2±0.1 pg (n=6-8), respectively.

Cells obtained from the four locations were structurally intact as judged under light microscopy, highly viable and exhibited low spontaneous histamine release (table 4.1). Mast cells obtained from the peritoneum, mesentery and lung were almost exclusively insensitive to formaldehyde fixation and stained positively with safranin. In contrast, those derived from the intestine were predominantly the formaldehyde sensitive, safranin negative type.

4.3.2 Anaphylactic histamine release

Mast cells obtained from the four anatomical locations released histamine in a dose-dependent fashion in response to stimulation with the specific allergen derived

from the nematode Nippostrongylus brasiliensis (fig 4.1) and anti-rat IgE (fig 4.2). In general, the peritoneal cells were the most responsive and the intestinal cells the least reactive. In each case, the release of histamine induced by the two immunological secretagogues was totally abolished by preincubation (20 min) of the cells in a glucose-free medium with the combined metabolic inhibitors antimycin A (1 μM) and 2-deoxyglucose (5 mM) (data not shown). In addition, anti-rat IgG also induced histamine release in a dose-related fashion from the four cell preparations with the peritoneal cells again the most reactive and the intestinal cells the least responsive (fig 4.3a).

4.3.3 Histamine release by lectins

Concanavalin A, a lectin which cross-links IgE by binding to sugar moieties in the Fc region, produced a dose-dependent release of histamine from all four cell preparations (fig 4.3b). The release was comparable for mast cells from the peritoneum and mesentery, decreased slightly for those from the lung while intestinal cells again showed the weakest reactivity. Moreover, the response in each case was suppressed at supramaximal concentrations of concanavalin A.

In contrast, only rat peritoneal mast cells responded to the lectin from wheat germ and the release was totally dependent on the presence of PS (15 μg/ml) (tables 4.2a and 4.2b). The tissue mast cells, with the exception of those from the mesentery which showed slight reactivity in the presence of the lipid, were essentially refractory to the histamine releasing action of the lectin.

4.3.4 Histamine release by calcium ionophores

The calcium ionophores A23187 (fig 4.4a), bromo A23187 (fig 4.4b), ionomycin (fig 4.5a) and chlortetracycline (fig 4.5b) all induced a pronounced secretion of histamine from rat peritoneal mast cells. The response to the first three agents was greatly suppressed at high concentrations. Significant but lower release was observed for the mesenteric and lung cells while those from the intestine were only weakly responsive to all four ionophores.

4.3.5 Histamine release by polybasic compounds and neuropeptides

The polyamine compound 48/80 (fig 4.6a) and the antibiotic polymyxin (fig 4.6b) were potent releasers of histamine from rat peritoneal mast cells. This effect was dramatically reduced for cells from the mesentery and to an even greater extent for those from the lung. Moreover, the two basic compounds were totally ineffective against the intestinal cell.

This pattern of graded responses was also observed for the neuropeptide substance P (fig 4.7a) and its synthetic analogue $SP_{1\text{-}4}C_{12}$ (fig 4.7b) and for the polyamino acids polylysine (fig 4.8a) and polyarginine (fig 4.8b). In slight contrast to compound 48/80 and polymyxin, these agents exhibited weak activity against the intestinal cell.

4.3.6 Histamine release by histamine receptor directed compounds

Rat peritoneal mast cells responded strongly to the H_2-agonist impromidine (fig 4.9a) and the H_2-antagonist ranitidine (fig 4.9b). Significant but lower release of histamine was also observed for the mesenteric cell while those derived from the lung and intestine were only weakly responsive to the secretory effect of the two agents. In contrast, dimaprit (fig 4.10), another H_2-agonist, was more specific in its mode of action, being a potent releaser of histamine from rat peritoneal cells while having essentially no effect on the other cell preparations studied.

4.3.7 Histamine release by other secretagogues

Rat peritoneal mast cells showed a weak release of histamine on treatment with the plasma substitute dextran but the response was greatly enhanced by the presence of PS (15 μg/ml) (tables 4.3a and 4.3b). In contrast, mast cells from the other three locations were only very weakly reactive or were essentially refractory to dextran both in the presence and absence of the lipid.

The detergents Triton X-100 (fig 4.11a) and to a lesser extent Tween-20 (fig 4.11b) were potent cytotoxic releasers of histamine from rat peritoneal, mesenteric,

lung and intestinal mast cells while N-formyl methionyl-leucyl-phenylalanine (FMLP), a synthetic bacterial peptide that activates both neutrophils and basophils, was totally ineffective against all four cell preparations studied (table 4.4).

4.4 Discussion

The present study has demonstrated that functional rat mast cells may be obtained by simple lavage of the peritoneal cavity and by enzymic dispersion of mesenteric, lung and intestinal tissues. Isolated cells from the four locations were highly viable, structurally intact as judged by light microscopy and exhibited a low spontaneous release of histamine. Moreover, these cells have been characterized in terms of their basic properties and in their functional responses to a number of immunological and non-immunological histamine liberators.

The bacterial proteolytic enzyme collagenase has long been employed in the isolation of tissue mast cells from a number of target organs [65,76,249-252]. Indeed, Pearce and Ennis [249], in an extensive study on the dispersive ability of a number of enzymes, found that collagenase was the most effective in the isolation of mast cells from mesenteric tissues of the rat and guinea pig. Moreover, they found that collagenase had one important additional advantage in that it is a highly specific protease and is not inhibited by serum or serum proteins. From this, it was then possible to carry out the entire dissociation in the presence of agents such as foetal calf serum and bovine serum albumin, thereby creating conditions which are known to have a pronounced stabilizing effect on mast cells.

In the present study, a near identical method using the enzyme collagenase was employed in the isolation of mast cells from the rat mesentery, lung and intestine. Cells from the former two locations, along with those from the peritoneum were well preserved by the fixatives Carnoy's and formol saline and stained metachromatically with safranin. In total contrast, those derived from the intestine were sensitive to formaldehyde fixation and stained orthochromatically with alcian blue. These variations in histochemistry therefore suggest the presence of distinct proteoglycans in the secretory granules. Indeed, Stevens et al [265] demonstrated that intestinal MMC from

rats infected with Nippostrongylus brasiliensis contain a protease-resistant, less highly sulphated proteoglycan named chondroitin sulphate di-B as opposed to heparin which is present in mast cells from the serosal cavities [89,90,257]. With this in mind, it seems probable that heparin might also be the predominant proteoglycan located in mast cell granules of the mesentery and lung.

This unique sensitivity of rat MMC towards formaldehyde-based fixatives has been further investigated by Enerbäck and his co-workers [256,261,328,332]. They found that good preservation of MMC could be obtained if the procedure was carried out in an isotonic, low concentration formaldehyde and acetic acid mixture. The effect of the ionic strength of the fixative is especially important and constitutes the so called 'critical electrolyte concentration' (CEC). Enerbäck proposed that MMC have a much lower CEC as compared to CTMC. Therefore, the matrix of MMC granules is more soluble in solutions of high salt concentrations than that of CTMC granules.

Mast cells obtained from the four locations released histamine in a dose-dependent fashion in response to stimulation with the specific allergen and anti-rat IgE. In each case, the peritoneal cells were the most responsive and the intestinal cells the least reactive. This difference in reactivity might reflect the number of IgE receptors present on the cell surface. Indeed, by employing purified intestinal mast cells, Befus, Lee and their co-workers [333,334] have demonstrated that these cells contained significantly fewer IgE receptors, in addition to having a lower receptor density, than the conventional peritoneal mast cells. Moreover, in a more recent study [335], these workers reported the existence of distinct IgE receptor types in purified populations of rat intestinal and peritoneal mast cells. The IgE receptors of the latter cells can be divided into two groups; low-affinity receptors with a relative molecular mass of 56,000 and high-affinity receptors that contain α-chains with a relative molecular mass of 51,000. In contrast, the corresponding α-chains of the high-affinity receptors in the intestinal cells are of a slightly higher relative molecular mass (59,000). These cells, in addition, possess two forms of low-affinity receptors; 50,000 and 58,000 respectively. It is then possible that these differences in receptor characteristics might be responsible for the fact that rat mast cells from different locations release different quantities of histamine in response to antigenic stimulation. Moreover, the above hypothesis might

also be applied to concanavalin A since the lectin is believed to cross-link IgE by binding to glucose and mannose residues in the carbohydrate portion of the immunoglobulin [50,297].

The lectin from wheat germ was more specific in its mode of action. In the present study, and in agreement with results of Ennis et al [336], the lectin was only active against mast cells from the peritoneum. The release was, however, totally dependent on the presence of PS. Tissue mast cells, with the exception of those from the mesentery which showed slight reactivity in the presence of the lipid, were essentially unresponsive. These findings may reflect differences in the membrane composition of the various mastocytes and it has been suggested that rat peritoneal mast cells may be naturally deficient in PS [337]. According to this view, the membranes of other mast cells are saturated with endogenous PS thus rendering them refractory to the potentiating effect of the lipid.

Although the role of IgE-antibody in anaphylactic histamine release in the rat is well established, there is less evidence for the involvement of IgG-antibodies. Moodley and Mongar [338] have shown that antisera to IgG subclasses 1, 2a, 2b and 2c were capable of releasing histamine from rat peritoneal mast cells of Lister hooded rats. In competition experiments designed to investigate whether secretion with anti-IgG subclasses was due to cross-reactivity with cell-bound IgE-antibody, these workers showed that only anti-IgG 2a exhibited an element of such reactivity.

More recently, peritoneal mast cells obtained from Sprague Dawley rats have been demonstrated to release histamine following challenge with whole anti-IgG serum [339]. The effect was found to be substantially blocked by preincubation of the cells with purified IgG. The results of the present study confirm part of the previous work and extend it to tissue mast cells from the rat mesentery, lung and intestine which were shown to release histamine, in varying degrees, following anti-IgG challenge.

Mast cells from the four locations responded in varying degrees to the histamine releasing action of the calcium ionophores A23187, bromo A23187, ionomycin and chlortetracycline. These results suggest that, in each case, an elevated level of the cation

in the cytosol is a necessary and sufficient trigger for secretion [19,136,149]. In the present study, the effects of the calcium ionophores were generally greatest against the peritoneal cell and weakest against the intestinal cell. The reasons for this difference are not obvious but may indicate that the control mechanism for the release process is different for each cell population. Alternatively, these observations may again reflect variations in the membrane compositions of the target cells, leading to differences in the lipid solubility and rate of diffusion of the ionophores.

The employment of polybasic compounds and neuropeptides in functional studies of mast cell heterogeneity has been well documented [279-281,298]. The differential effects of these agents in relation to species heterogeneity have already been discussed in chapter three. Moreover, the present study indicates that this phenomenon also extends to different mast cell populations from the rat. Mast cells from the peritoneum released a significant amount of their histamine in response to stimulation with the basic agents compound 48/80, polymyxin, substance P, $SP_{1\text{-}4}C_{12}$, polylysine and polyarginine. In contrast, enzymically dispersed mast cells of the mesentery, lung and intestine show a continuous gradation in reactivity towards these compounds. Moreover, it has been suggested [280,281] that basic agents exert their effects through a common, non-specific 'receptor' or binding site. With this in mind, it is possible that tissue mast cells of the rat have a reduced number or are deficient in these 'receptors'.

In accordance with the findings of Lau and Pearce [340-342], the H_2-receptor directed compounds dimaprit, impromidine and ranitidine were potent releasers of histamine from rat peritoneal mast cells. The release in each case was extremely rapid and blocked by metabolic inhibitors and extremes of temperature [342]. Moreover, the release induced by these H_2-ligands was independent of extracellular calcium and also blocked by BDTA [342]. The latter is putatively an inhibitor of the so-called polyamine receptor [343] and, as such, dimaprit, impromidine and ranitidine may induce histamine release by virtue of their basic character and through a mechanism similar to that of other basic compounds such as compound 48/80. In keeping with the above hypothesis, these H_2-ligands were also much less reactive against tissue mast cells of the same animal.

The detergents Triton X-100 and Tween-20 belong to a unique group of mast cell secretagogues termed non-selective releasers [53]. These agents are cytotoxic and act by disruption of the mast cell membrane, thereby liberating all of the intracellular contents including histamine. In keeping with this mechanism, the two agents were potent releasers of histamine from rat mast cells from the peritoneum, mesentery, lung and intestine.

Dextran is a branched chain homopolymer of α-D-glucopyranosyl residues and has been clinically employed as a plasma substitute and blood volume expander [62,299,302]. Parenteral adminstration of this compound produces an acute anaphylactoid reaction in the rat [344] but is completely without effect in any other species including the mouse, hamster, guinea pig, rabbit, pigeon and dog [299,302]. The systemic reaction in the rat is accompanied by a striking elevation in the level of plasma histamine and a substantial degranulation of mast cells in those areas in which the inflammatory response is most marked [344].

The specific action of dextran is also manifest in vitro. The polysaccharide, in the presence of PS, is an effective releaser of histamine from serosal mast cells of the rat [278,302,345]. However, it is completely inactive against pulmonary, colonic and mesenteric mast cells of the guinea pig and man and against peritoneal cells of the hamster and mouse [76,250,251,346,347]. Moodley et al [348] proposed that dextran appears to produce its effects on responsive mast cells by interaction with specific glucoreceptors on the cell membrane. The present study confirms the effect of dextran on rat peritoneal mast cells and extends it to tissue mast cells from the same animal which, in total contrast, were only very weakly responsive or were essentially refractory to the histamine releasing action of this agent.

A number of synthetic N-formyl methionyl dipeptides and tripeptides, of which one of the most potent so far prepared is N-formyl methionyl-leucyl-phenylalanine (FMLP), are thought structurally to resemble certain naturally occurring bacterial chemotaxins. These peptides are strongly chemotactic for mammalian neutrophils and macrophages and induce lysosomal enzyme secretion from the former cell type in the presence of cytochalasin B. They also evoke histamine release from human basophils

and this effect correlates closely with their chemotactic activity [349]. It has been suggested that formylmethionyl peptides may constitute part of the defence mechanism against bacterial infection. Local release of the peptide at tissue sites of bacterial invasion would liberate histamine which, by increasing capillary permeability, could facilitate the recruitment and activation of phagocytic cells. The present study has shown that in spite of its activity on human basophils, FMLP was inactive on both peritoneal and tissue mast cells of the rat.

The present investigation clearly demonstrates the existence of functional heterogeneity in rat mast cells derived from different anatomical locations. Moreover, it also shows the danger in the usage of the terms MMC and CTMC. These had been originally employed in the description of the two distinct mast cell types in the gastrointestinal tract but have now been extended far beyond their original meaning. Indeed, these terms have been used to divide rat mast cells into two categories based on their locations. Clearly, this is both incorrect and misleading since mast cells from different connective tissues of the rat clearly exhibit striking variations in their functional responsiveness.

Fig 4.1 Histamine release induced by worm allergen from actively sensitized rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN) (n=4-9).

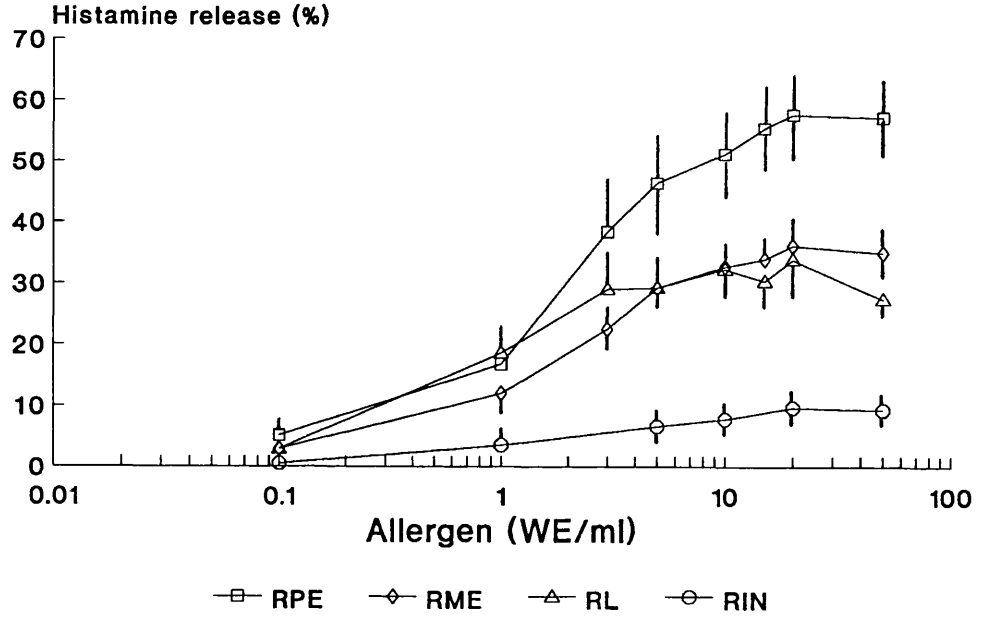


Fig 4.2 Histamine release induced by anti-rat IgE from isolated rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN) (n=4-8).

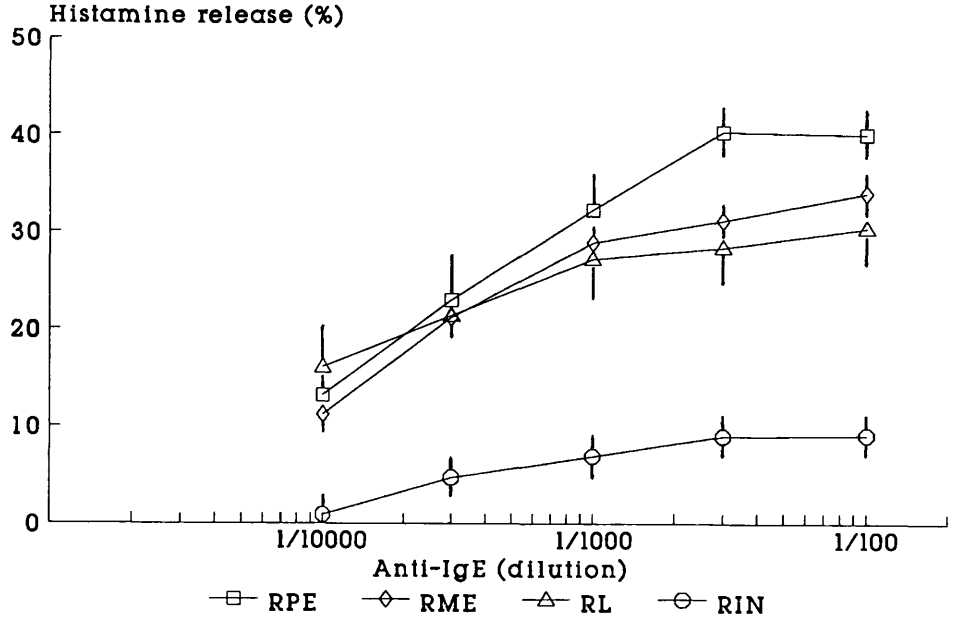


Fig 4.3 Histamine release induced by (a) anti-rat IgG (n=4) and (b) concanavalin A (n=4-6) from isolated rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN).

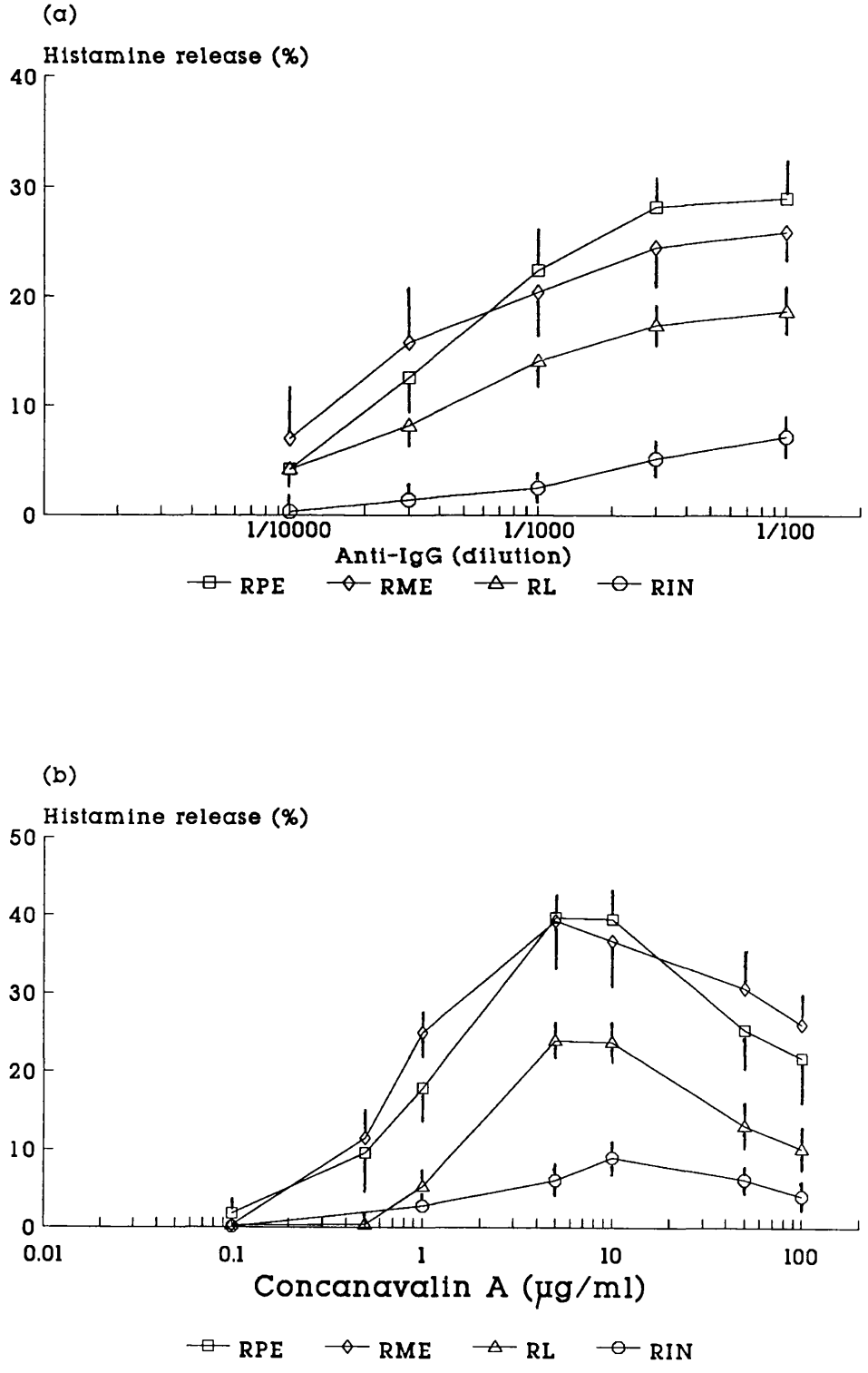
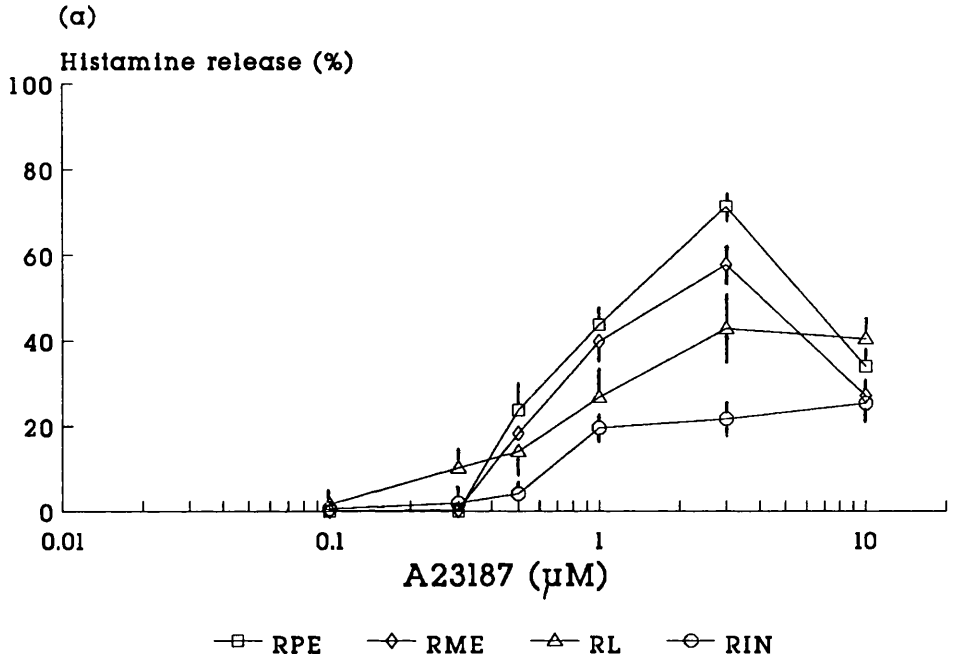


Fig 4.4 Histamine release induced by (a) A23187 (n=4-5) and (b) bromo A23187 (n=4-5) from isolated rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN).



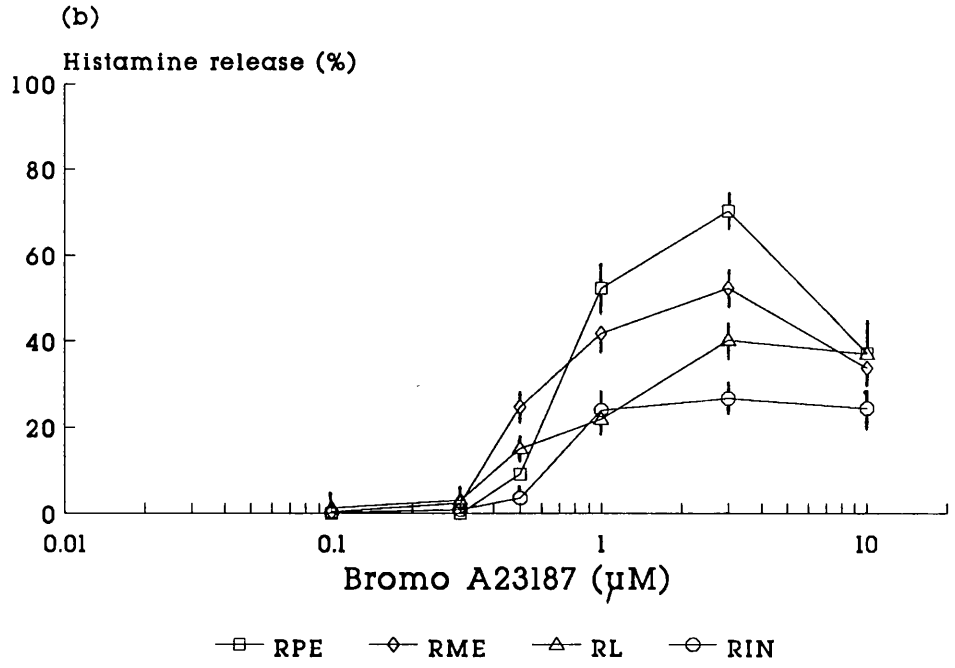
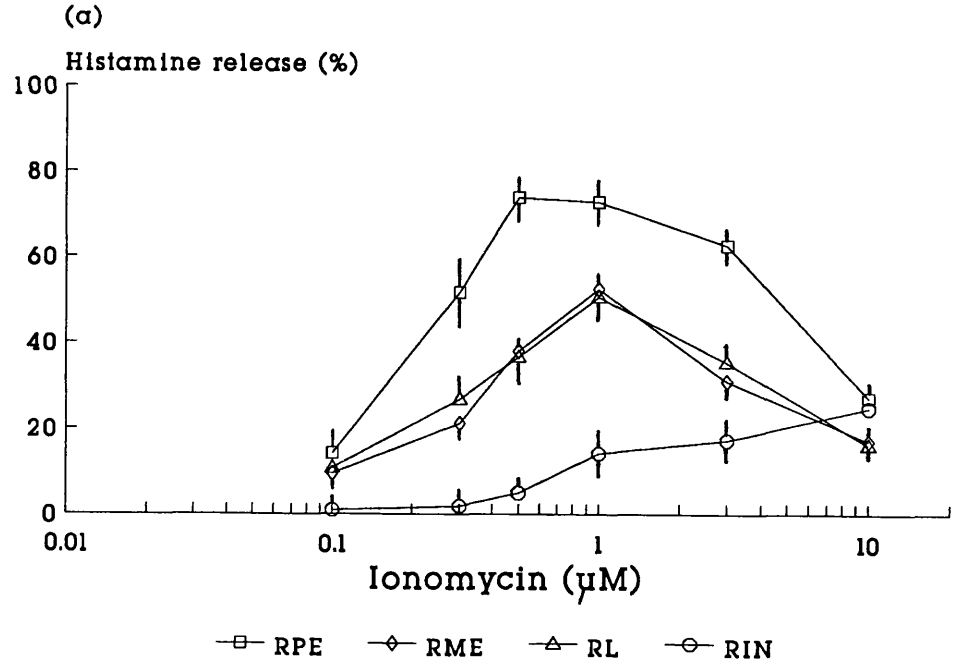


Fig 4.5 Histamine release induced by (a) ionomycin (n=4-5) and (b) chlortetracycline (n=4) from isolated rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN).



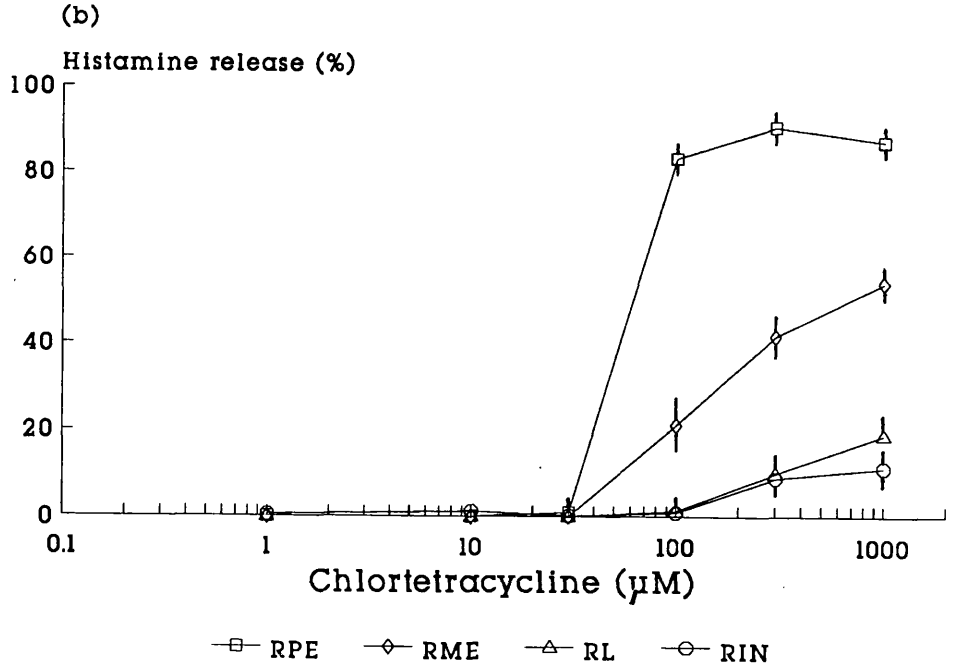
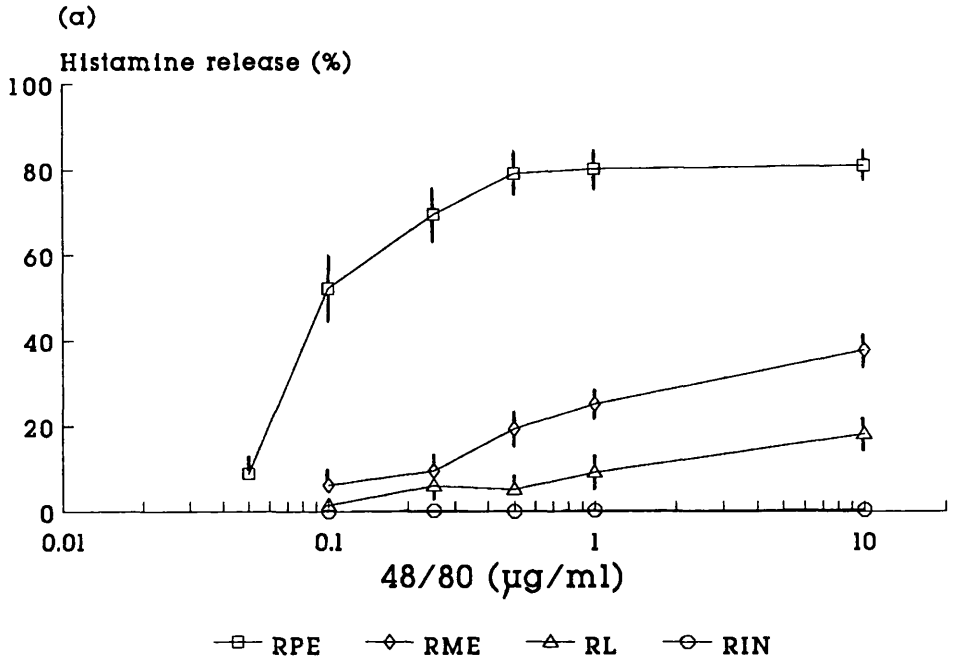


Fig 4.6 Histamine release induced by (a) compound 48/80 (n=3-5) and (b) polymyxin (n=3-7) from isolated rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN).



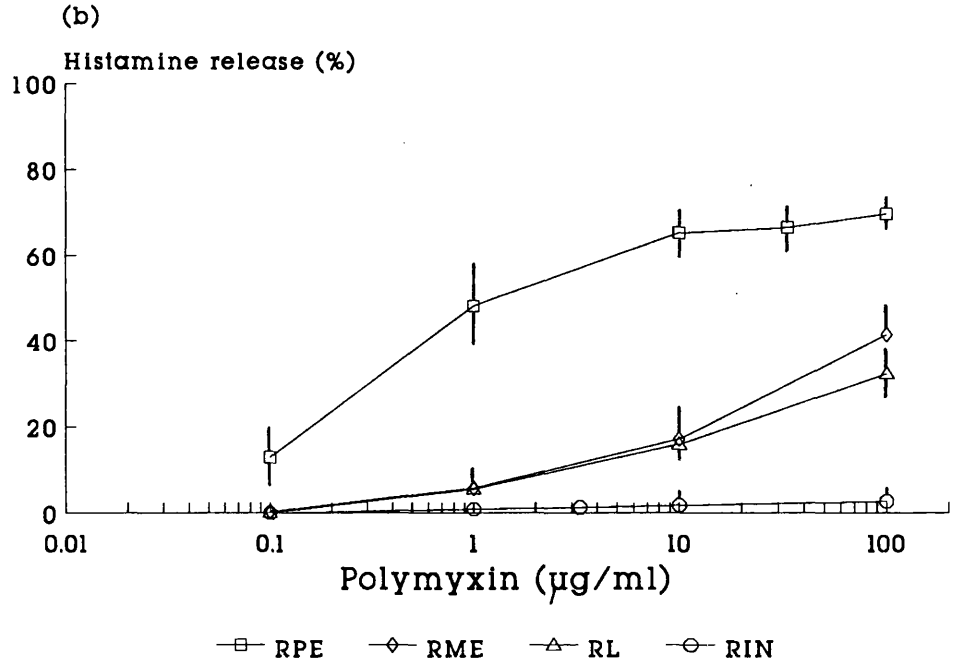
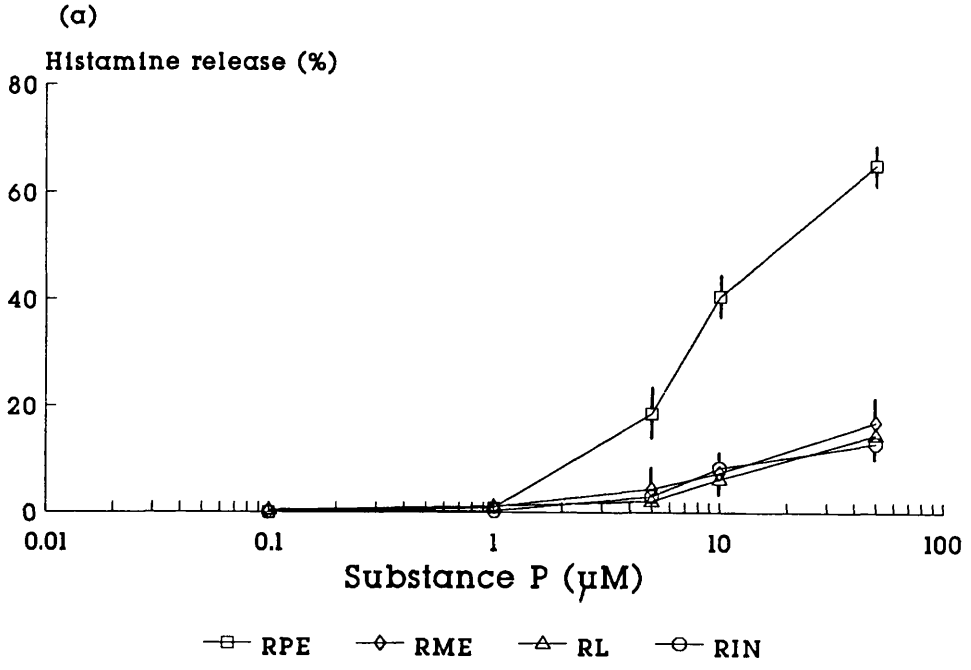


Fig 4.7 Histamine release induced by (a) substance P (n=4-7) and (b) $SP_{1-4}C_{12}$ (n=4-9) from isolated rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN).



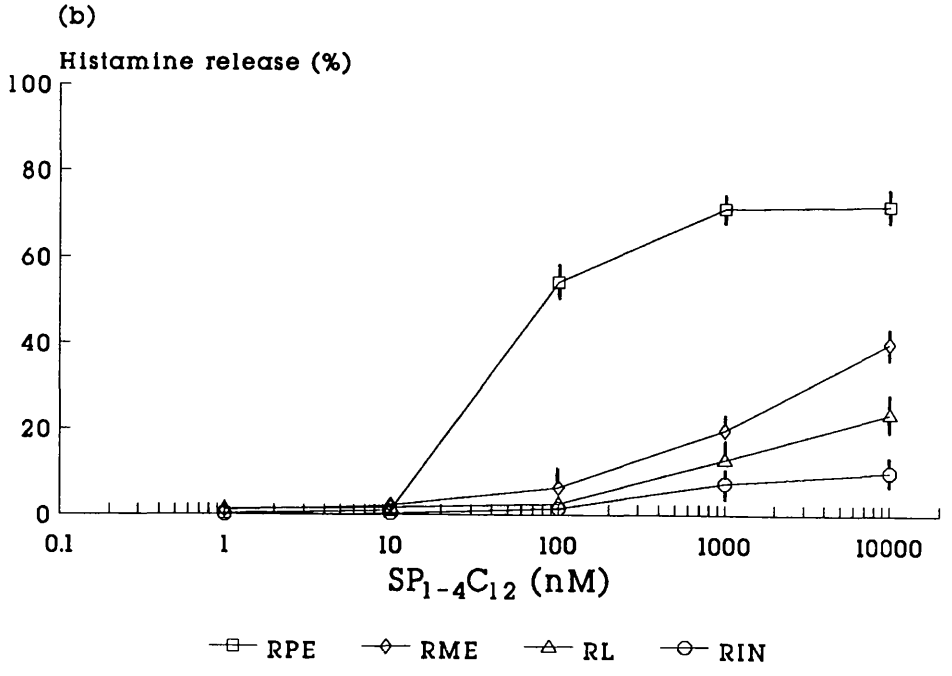
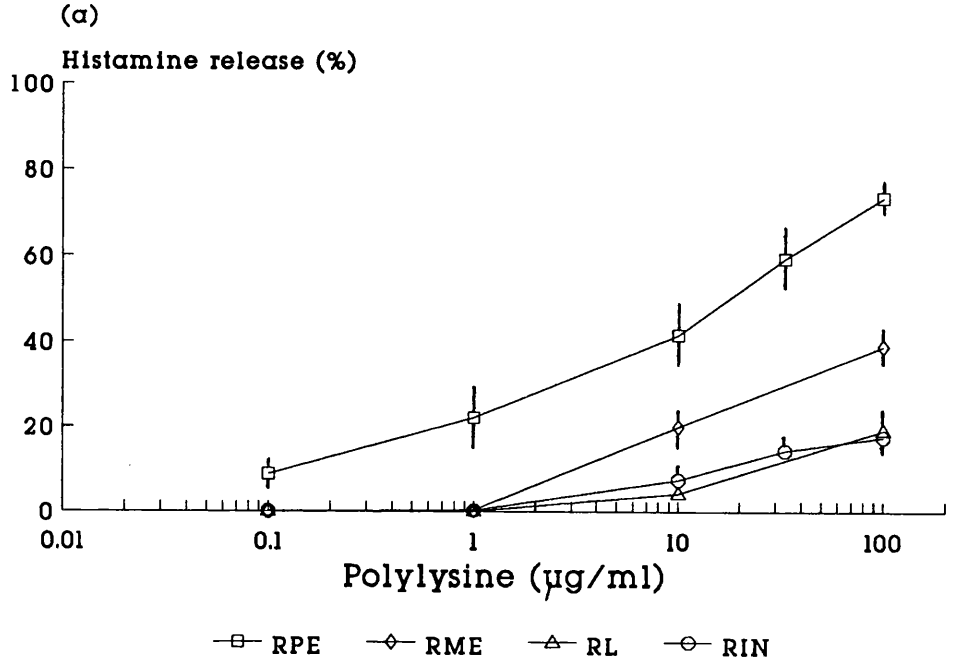


Fig 4.8 Histamine release induced by (a) polylysine (n=4-7) and (b) polyarginine (n=4-7) from isolated rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN).



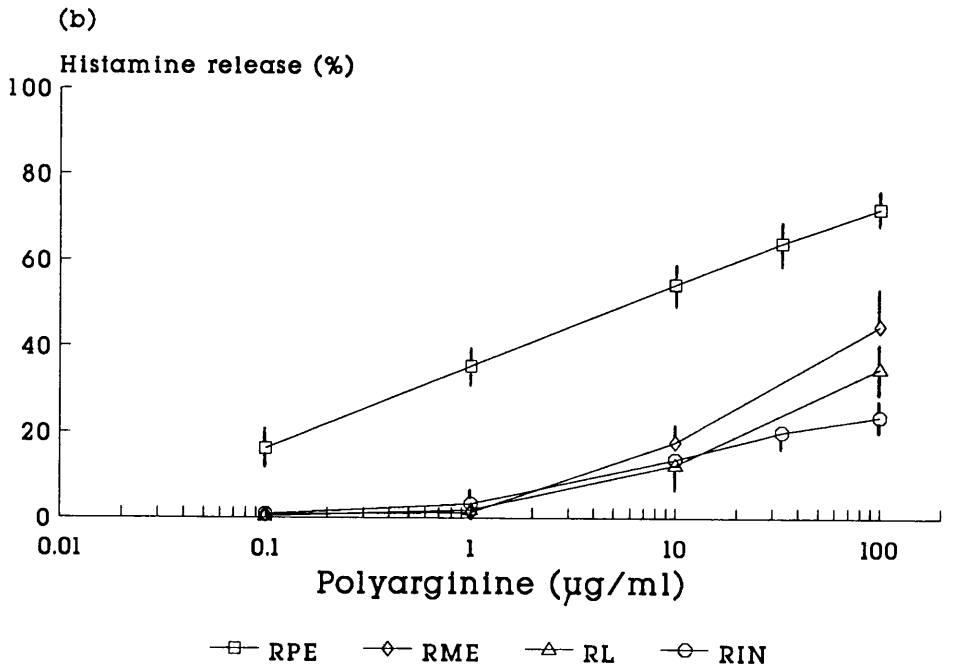
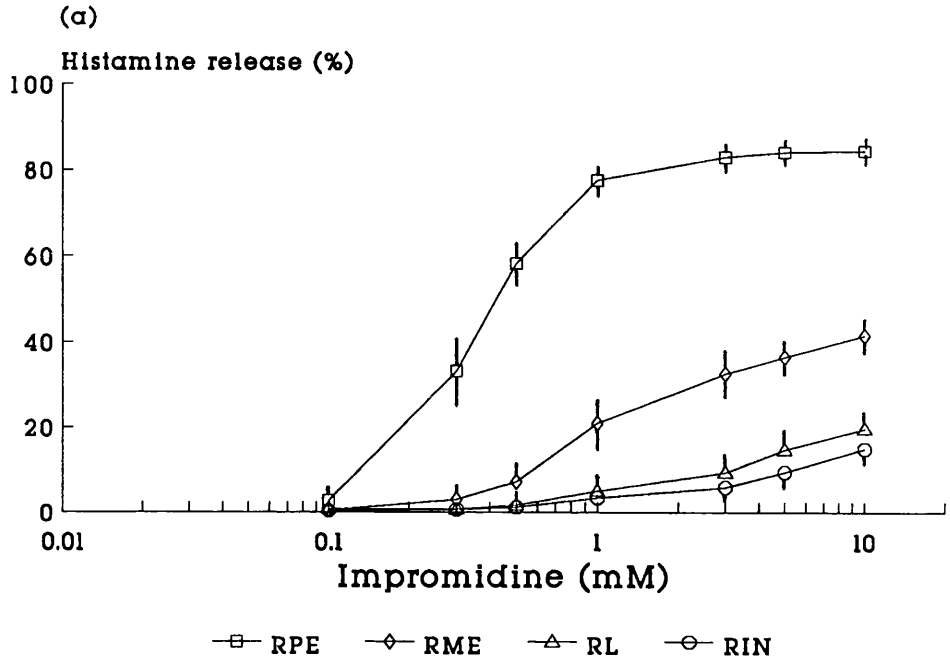


Fig 4.9 Histamine release induced by (a) impromidine (n=4) and (b) ranitidine (n=4-5) from isolated rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN).



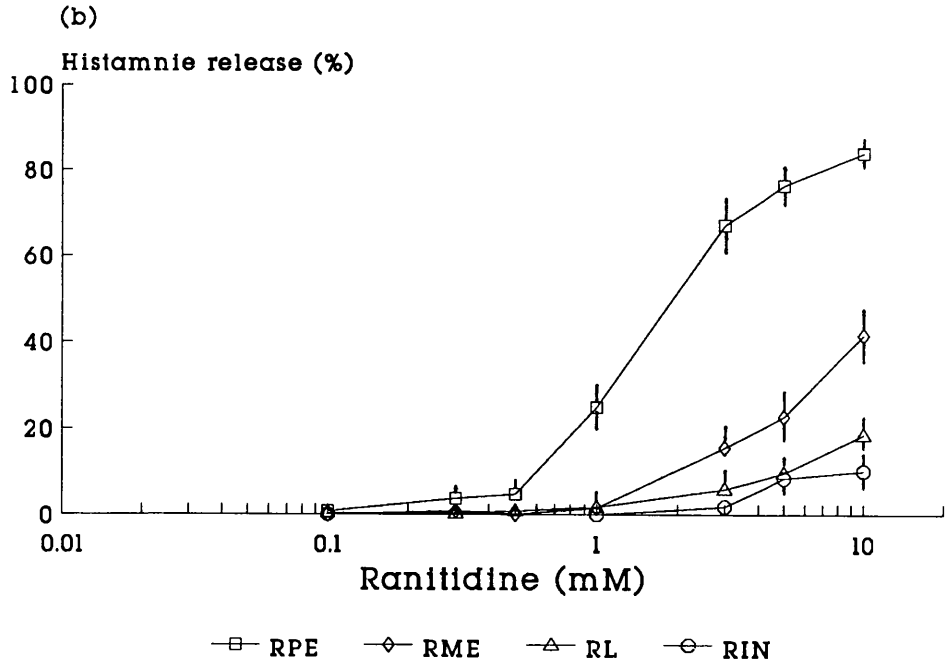


Fig 4.10 Histamine release induced by dimaprit from isolated rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN) (n=4).

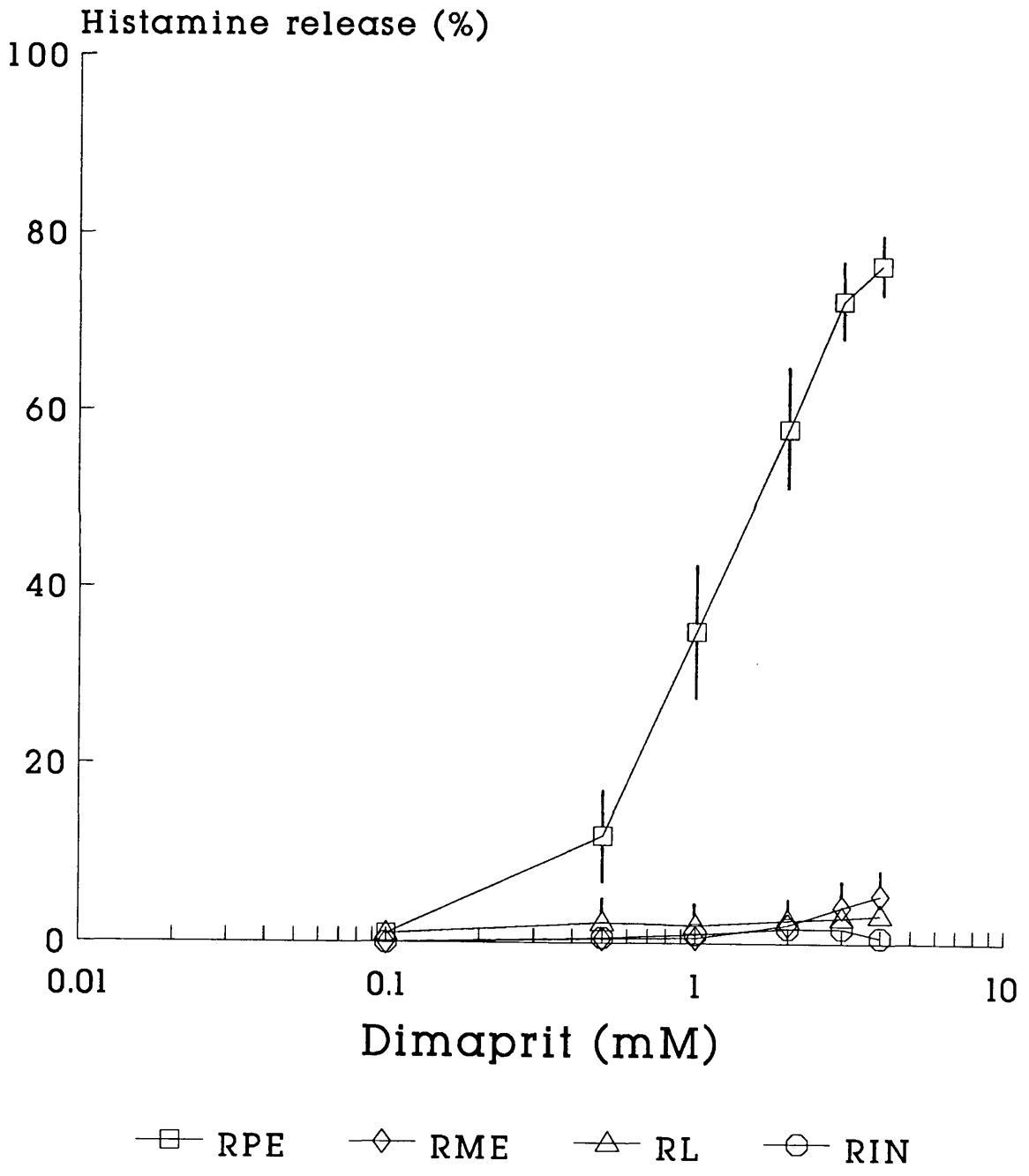
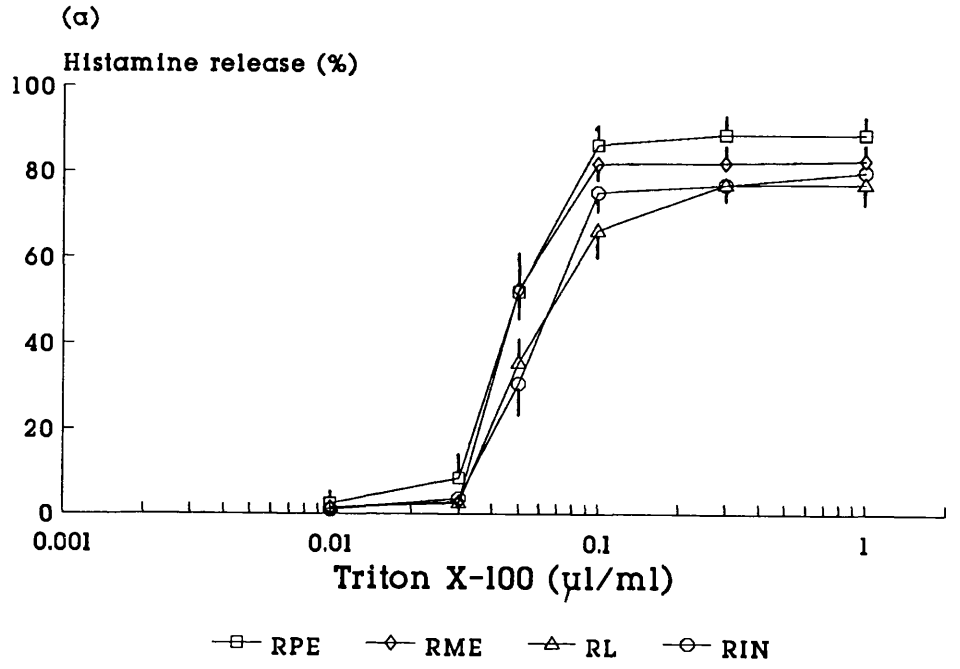


Fig 4.11 Histamine release induced by (a) Triton X-100 (n=4) and (b) Tween-20 (n=4) from isolated rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN).



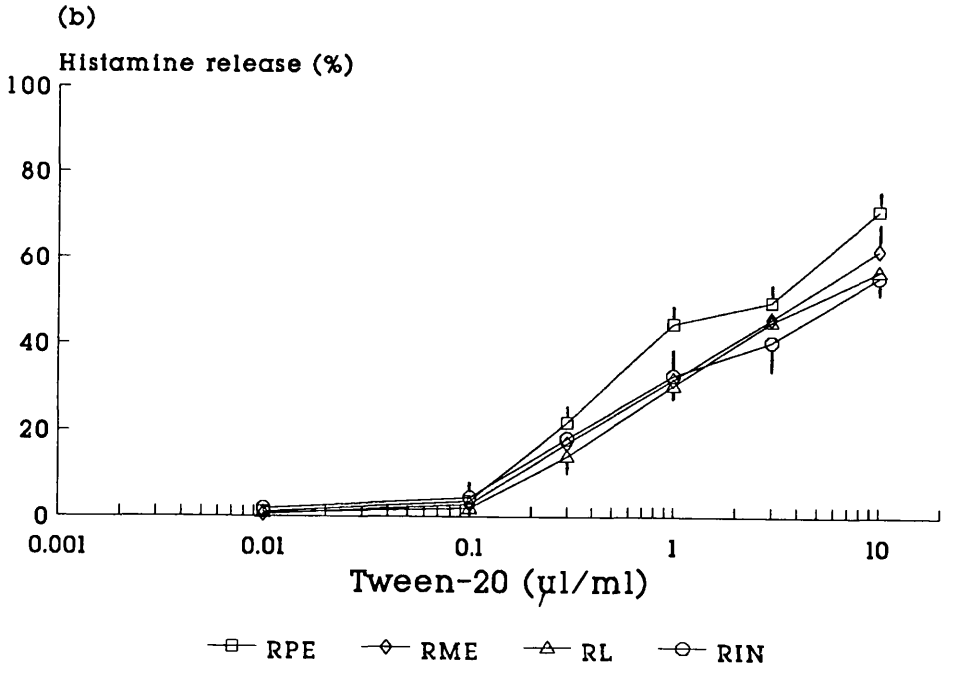


Table 4.1 Some basic properties of rat mast cells isolated from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN).

	RPE n=8	RME n=6	RL n=6	RIN n=6
Dispersion efficiency (%)	-	27.4±2.1	23.3±2.0	14.7±1.7
Mast cells (% total)	5.3±0.6	4.2±0.7	3.5±0.6	12.7±1.9
Viable cells (% total)	98.1±0.7	88.3±1.9	82.1±2.5	68.4±3.1
Histamine (pg/mast cell)	24.3±2.3	7.6±0.8	3.8±0.3	1.2±0.1
Spontaneous histamine release (%)	6.2±0.4	7.2±0.7	6.5±1.1	8.8±0.3
Formaldehyde sensitive (%)	0.4±0.2	1.1±0.6	1.2±0.7	96.1±1.6
Safranin positive (%)	99.7±0.1	99.0±0.5	99.5±0.6	0.3±0.1

Table 4.2 Histamine release from rat mast cells induced by wheat germ lectin in the absence (a) or presence (b) of phosphatidylserine (15 μg/ml).

(a)

Concentration (μg/ml)	Histamine release (%) RPE n=4	RME n=4	RL n=4	RIN n=4
100	1.7±0.6	0.9±0.3	0.8±0.4	1.0±0.3
50	1.4±0.8	-0.1±0.2	0.1±0.2	0.5±0.3
10	1.0±0.4	0.1±0.4	0.1±0.4	0.9±0.0
5	0.9±0.5	-0.1±0.1	-0.1±0.2	0.2±0.2
1	-0.1±0.4	0.1±0.3	-0.2±0.4	-0.1±0.3

(b)

Concentration (μg/ml)	Histamine release (%) RPE n=4	RME n=4	RL n=4	RIN n=4
100	45.5±3.8	10.6±1.2	3.7±1.0	1.5±0.3
50	34.6±3.4	6.6±0.6	1.5±0.3	1.4±0.3
10	25.3±3.4	3.1±0.5	1.4±0.3	1.3±0.8
5	16.2±1.7	1.2±0.4	0.7±0.1	0.6±0.4
1	10.0±1.5	0.7±0.5	0.1±0.3	0.6±0.4

Table 4.3 Histamine release from rat mast cells induced by clinical dextran in the absence (a) or presence (b) of phosphatidylserine (15 μg/ml).

(a)

Concentration (μg/ml)	Histamine release (%) RPE n=4	RME n=4	RL n=4	RIN n=4
10	12.9±1.6	3.1±0.4	1.7±0.2	0.9±0.1
3	7.2±0.7	2.0±0.2	0.9±0.2	0.3±0.1
1	3.3±0.7	0.8±0.3	0.4±0.1	0.4±0.2
0.3	1.6±0.4	0.5±0.1	0.3±0.2	0.0±0.2
0.1	0.9±0.4	0.3±0.2	0.0±0.1	-0.4±0.2

(b)

Concentration (μg/ml)	Histamine release (%) RPE n=4	RME n=4	RL n=4	RIN n=4
10	43.3±2.6	10.1±1.4	5.5±1.0	1.3±0.2
3	34.2±2.5	5.3±0.8	2.0±0.4	0.7±0.1
1	27.9±3.6	1.9±0.3	0.9±0.1	0.1±0.2
0.3	9.0±2.1	1.4±0.1	0.6±0.1	0.4±0.1
0.1	2.4±0.5	1.1±0.1	0.4±0.1	-0.2±0.2

Table 4.4 Histamine release from rat mast cells induced by f-met-leu-phe.

Concentration (μM)	Histamine release (%) RPE n=4	RME n=4	RL n=4	RIN n=4
1000	0.0±0.2	-0.4±0.6	0.4±0.2	0.6±0.1
300	-0.2±0.2	-0.2±0.6	1.0±0.6	0.4±0.2
100	-0.2±0.3	-0.6±0.4	0.5±0.7	0.3±0.2
30	-0.3±0.4	0.5±0.3	0.1±0.3	0.3±0.3
10	0.0±0.1	-0.9±0.4	-0.2±0.4	0.2±0.2
3	-0.2±0.1	-0.5±0.3	-0.6±0.3	0.2±0.3
1	0.0±0.1	0.8±0.4	-0.7±0.3	0.0±0.3

CHAPTER FIVE

MAST CELL HETEROGENEITY IN THE RAT: EFFECTS OF ANTI-ALLERGIC COMPOUNDS

5.1 Introduction

Mast cells and basophils are involved, through their high affinity IgE receptors, in allergic or immediate hypersensitivity type reactions. Indeed, the widespread involvement of mastocytes in a diversity of allergic disorders has naturally led to attempts to develop drugs that suppress mast cell function. Such drugs would represent an obvious therapeutic development since agents that prevent the release of inflammatory mediators should provide a means of controlling the earliest stages of the allergic response. However, there are a number of problems inherent in this approach, not least being the recognition that many allergic disorders are complex inflammatory processes involving a variety of different cell types. More relevantly for the present study, however, is the fact that many potential anti-allergic compounds show a high degree of mast cell selectivity in their action; a phenomenon that is best typified by the clinically used, anti-asthmatic drug disodium cromoglycate [257,279,283,299,300,302]. Moreover, the above compound has been commonly used as a functional marker in the differentiation of rat intestinal mucosal mast cells and their connective tissue counterparts [330,350].

The findings of chapter four clearly demonstrate that rat mast cells from different locations are functionally heterogeneous in their responses to a number of histamine liberators. It is therefore the aim of the present study to examine whether this phenomenon also extends to agents that may modulate histamine release.

5.2 Methods

All methods used in this study were as described in chapter two.

5.3 Results

Unless otherwise stated, histamine release in all cases was induced from sensitized cells by specific allergen derived from the nematode, Nippostrongylus brasiliensis (see section 2.11.3).

5.3.1 Effects of DSCG and related compounds

DSCG (fig 5.1) and its more recently introduced congener nedocromil sodium (fig 5.2) produced a comparable dose-dependent inhibition of allergen-induced histamine release from rat peritoneal mast cells. Both drugs, however, exhibited a sharp tachyphylaxis, and activity was rapidly lost on preincubation with the cells before challenge (figs 5.3 and 5.4). In contrast, the chromones were only very weakly active against tissue mast cells derived from the mesentery, lung and intestine (figs 5.1 and 5.2). Moreover these effects were unaltered upon preincubation (tables 5.1 and 5.2).

Quercetin, a naturally occurring flavonoid structurally related to DSCG, was a potent inhibitor of allergen-induced histamine release from both peritoneal and tissue mast cells of the rat (fig 5.5). Another flavonoid, chrysin, also exhibited comparable activity on all four cell preparations but the effects observed were much less pronounced than in the case of quercetin (fig 5.6).

5.3.2 Effects of LU 48953 and MY 1250

LU 48953 (Knoll) and MY 1250 (Bayer) are two new, potential anti-allergic compounds. Similarly to the chromones, the two agents suppressed histamine release from rat peritoneal mast cells in a dose-dependent manner (figs 5.7 and 5.8) with tachyphylaxis again a prominent feature (figs 5.9 and 5.10). In contrast, both LU 48953 and MY 1250 were essentially ineffective against tissue mast cells from the mesentery, lung and intestine, with or without initial preincubation (tables 5.3 and 5.4).

5.3.3 Effects of cAMP-active compounds

The cyclic nucleotide analogue Bu_2cAMP (fig 5.11) and the phosphodiesterase inhibitors, theophylline (fig 5.12) and 3-isobutyl-1-methylxanthine (IBMX) (fig 5.13) blocked histamine release in a dose-related fashion from mast cells derived from the rat peritoneum, mesentery, lung and intestine. The tissue cells were slightly less sensitive towards the inhibitory activity of the former compound. In contrast, the β-adrenoceptor agonists, isoprenaline and salbutamol, were essentially ineffective against all four cell preparations studied (tables 5.5 and 5.6).

5.3.4 Effects of cimetidine

The H_2-receptor antagonist cimetidine inhibited histamine secretion from rat peritoneal mast cells in a concentration-dependent manner (fig 5.14). The compound also showed comparable activity against cells from the mesentery and lung whereas those from the intestine exhibited a much lower responsivity.

5.4 Discussion

In keeping with their differential responsiveness towards various histamine liberators of both immunological and non-immunological nature, the present study has extended the functional heterogeneity of rat mast cells from various locations to the effects of a number of anti-allergic compounds.

The selective effect of DSCG and nedocromil sodium on isolated mast cells from different species has already been clearly demonstrated in chapter three. In the present study, this functional heterogeneity also applied to rat mast cells from different anatomical locations. The chromones were potent inhibitors of allergen-induced histamine release from mast cells of the peritoneum. This effect is in accordance with the in vivo observation that DSCG is an effective suppressor of passive peritoneal anaphylactic reactions in the rat [320,351]. In the present study, mast cells from the mesentery and lung were only very weakly sensitive to the inhibitory effect of the chromones. These results are surprising in view of the observations that DSCG is an effective blocker of respiratory anaphylaxis in the rat [321] and that the same compound is capable of inhibiting histamine release from fragments of chopped lung

[352]. It can be argued that the lower response of both mesenteric and lung mast cells to the chromones may be attributed to the isolation procedure. However, this suggestion is rather doubtful since peritoneal mast cells undergoing the same isolation procedure exhibited only a very slight reduction in response as compared to untreated cells (data not shown). Moreover, initial experiments on fragments of chopped lung have failed to repeat the dramatic effect observed by Sheard and Blair [352]. In agreement with various published reports [257,279,283,302,330,350], both DSCG and nedocromil sodium were essentially ineffective in inhibiting allergen-induced histamine release from rat intestinal mast cells.

The action of DSCG as an inhibitor of histamine release from mast cells has been the subject of intense research. Moreover, it has been shown that the drug is most effective when added together with the histamine releasing stimulus [283,353,354]. This is somewhat unusual since many inhibitory drugs require preincubation with the cells in order to manifest their maximal effect. The converse is true for the chromones, where the degree of inhibition of histamine release decreases with increasing preincubation time before the addition of the stimulus. However, more recent studies have shown that this tachyphylactic phenomenon only applies to certain mast cell populations, notably those derived from the rat serosal cavities [283,353,354] and human lung parenchyma [76,284,285,323], whereas other mast cell populations such as those from human colonic mucosa [76] and BAL [284,285,323] are unaffected by an initial preincubation with the chromones. In the present study, tachyphylaxis on rat peritoneal mast cells was also observed for the two new anti-allergic compounds LU 48953 and MY 1250, suggesting that they may act via a similar mechanism to the chromones.

Although widely used in research and in the prophylaxis of allergic conditions, the mechanism of action of the chromones remains unclear. DSCG has been by far the most extensively studied and was initially thought to exert its effect through sequestration of extracellular calcium [355], thereby preventing secretion which is a calcium-dependent process. This hypothesis is unlikely as DSCG is active in the micromolar concentration range against rat mast cells, whereas extracellular calcium is present at 1000 times this concentration. Also, sequestration of extracellular calcium

by DSCG could not account for its inhibition of non-immunological induced histamine release [356], a process that is independent of the presence of this cation in the extracellular medium.

Roy and Warren [357] have shown that, in vitro, DSCG is an inhibitor of cAMP phosphodiesterase. However, the IC_{50} value for this compound in inhibiting total activity of the above enzyme is approximately 10-fold higher than its IC_{50} in blocking mediator secretion from mast cells, suggesting this mechanism to be improbable in vivo. Most attention has recently been focused on the association of DSCG with a cell surface receptor. A DSCG binding site has been demonstrated on rat basophilic leukaemia cells (RBL-2H3) [358], and further studies have suggested that this protein is involved in calcium gating following activation [359]. However, the possible involvement of a DSCG receptor has been questioned by the fact that, although DSCG binds to RBL-2H3 cells, it is a very weak inhibitor of histamine release in this system. Moreover, the RBL cell has been the sole cell line in which the DSCG binding protein has been identified.

The inhibitory effect of DSCG, notably in rat peritoneal mast cells, has been associated with phosphorylation of a protein with a relative molecular mass of 78,000 [239,241]. Activation of rat mast cells with anti-IgE, compound 48/80 or the calcium ionophore A23187 results in the rapid phosphorylation, via a calcium-dependent reaction, of proteins with relative molecular masses of 42,000, 59,000, 68,000 and 78,000 [239-241]. The phosphorylation of the former three proteins can be observed at a time when histamine release is starting; however, phosphorylation of the 78,000 protein is much slower and occurs after cell secretion. It has been demonstrated that DSCG induces phosphorylation of this protein [239] and its decay parallels tachyphylaxis, thereby suggesting that the 78,000 protein may be responsible for the effects of DSCG. More recently, the action of this compound has been linked to protein kinase C (360,361), thereby suggesting an alternative role for DSCG in protein phosphorylation.

The naturally occurring plant flavonoid, quercetin, is structurally related to DSCG and, along with a number of other flavonoids, has been demonstrated to inhibit

histamine release from mast cells and basophils [331,362,363]. In the present study, quercetin has been shown to exhibit a considerably broader spectrum of activity than DSCG. Indeed, apart from being a potent inhibitor of histamine release from rat peritoneal, mesenteric and lung mast cells, the compound, in agreement with the results of Pearce et al [331], also exhibited similar activity against mast cells from the intestine. Moreover, in further contrast to DSCG in which tachyphylaxis is a prominent feature, preincubation of rat peritoneal mast cells with quercetin does not change the inhibition profile produced by this compound [364,365]. The other flavonoid employed in this study, chrysin, also exhibited comparable inhibitory activity against all four cell preparations but the effect observed was much less pronounced than that seen with quercetin. Finally, the precise mechanism whereby flavonoids prevent histamine release is still the subject of continuing research, but it has been shown that these compounds are capable of inhibiting a number of key enzymes involved in secretion such as Ca-ATPase, cyclic nucleotide phosphodiesterases, cyclooxygenase, lipoxygenase, phospholipase A_2 and protein kinase C [362-366].

It has generally been appreciated that agents which elevate intracellular concentrations of cAMP are capable of inhibiting mediator release from the mast cell. In vitro, a rise of cAMP may be achieved by a number of methods, namely by application of appropriate analogues of the nucleotide, by activation of adenylate cyclase with cholera toxin, certain prostaglandins or sympathomimetic amines, or by preventing the breakdown of the nucleotide by inhibition of phosphodiesterase with methylxanthines and related compounds. Of these agents, sympathomimetic amines and methylxanthines are used therapeutically in the treatment of allergic conditions such as asthma. They owe their clinical efficacy largely to their bronchodilator activity but their ability to prevent mediator release from the mast cell may also contribute to their utility.

However, in rat mast cells, the relationship between an elevation in cAMP levels and inhibition of mediator release is not as clear cut as in other cell systems. The methylxanthine theophylline, a potent inhibitor of histamine release from rat peritoneal mast cells [221-223,330], was previously found to be totally ineffective against intestinal mucosal mast cells from the same animals [330]. Moreover, β-adrenoceptor

agonists such as isoprenaline, despite raising cAMP levels, were reported to be inactive against rat mast cells from various locations [219-221,257]. The findings in the present study confirm the inconsistent effects of cAMP-active agents. Histamine release from the rat peritoneum, mesentery and lung was inhibited by the cyclic nucleotide analogue, Bu_2cAMP, and by the phosphodiesterase inhibitors, theophylline and IBMX. In contrast to the results of Pearce et al [330], these agents also exhibited comparable activity against the intestinal cell. The reasons for this discrepancy are not obvious, but may reflect the use of lower control releases (8.4±2.3 % as compared to 27.7±3.4 %) or the employment of a different strain of rats (Sprague Dawley as compared to Wistar Furth).

In accordance with results in the literature [219-221,257] concerning the effects of β-adrenoceptor agonists, both isoprenaline and salbutamol were ineffective in inhibiting histamine release from rat peritoneal mast cells. Tissue mast cells from the mesentery, lung and intestine were similarly insensitive to the two agents. The inability of β-adrenoceptor agonists to inhibit histamine release from rat mast cells may be attributed to a lack of coupled, functional β-adrenoceptors in these cells or to the generation of cAMP in discrete pools not directly linked to the inhibitory mechanism [136,219,224].

The H_2-receptor antagonist cimetidine, used clinically in the treatment of peptic ulcer disease, inhibited histamine secretion from rat peritoneal mast cells in a dose-dependent fashion. The compound also showed comparable activity against mast cells from the mesentery and lung whereas those from the intestine exhibited a much lower responsivity. It has been proposed [342,367] that the inhibitory action of histamine receptor directed ligands is due to the intercalation of the compounds into the lipid bilayer in such a way as to stabilize the cell membrane, thereby preventing the functional changes involved in exocytosis. Indeed, the lower sensitivity of the intestinal mast cells towards cimetidine may indicate a difference in the constitution of the membrane as compared to the other cell populations.

In total, the present study has reinforced the findings of chapter four in that rat mast cells derived from different anatomical locations are functionally heterogeneous. Moreover, it further emphasised the danger in the simple classification of these cells

into two categories (MMC and CTMC) based on their histochemical properties. Clearly, mast cell heterogeneity in the rat is much more complex than this basic division.

Fig 5.1 Effect of disodium cromoglycate (DSCG) on allergen-induced histamine release from actively sensitized rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN). The drug was added simultaneously with stimulus to cells. The control releases were 29.9±5.2 %, n=5, RPE; 30.8±3.3 %, n=6, RME; 23.5±3.7 %, n=5, RL and 9.4±1.6 %, n=5, RIN.

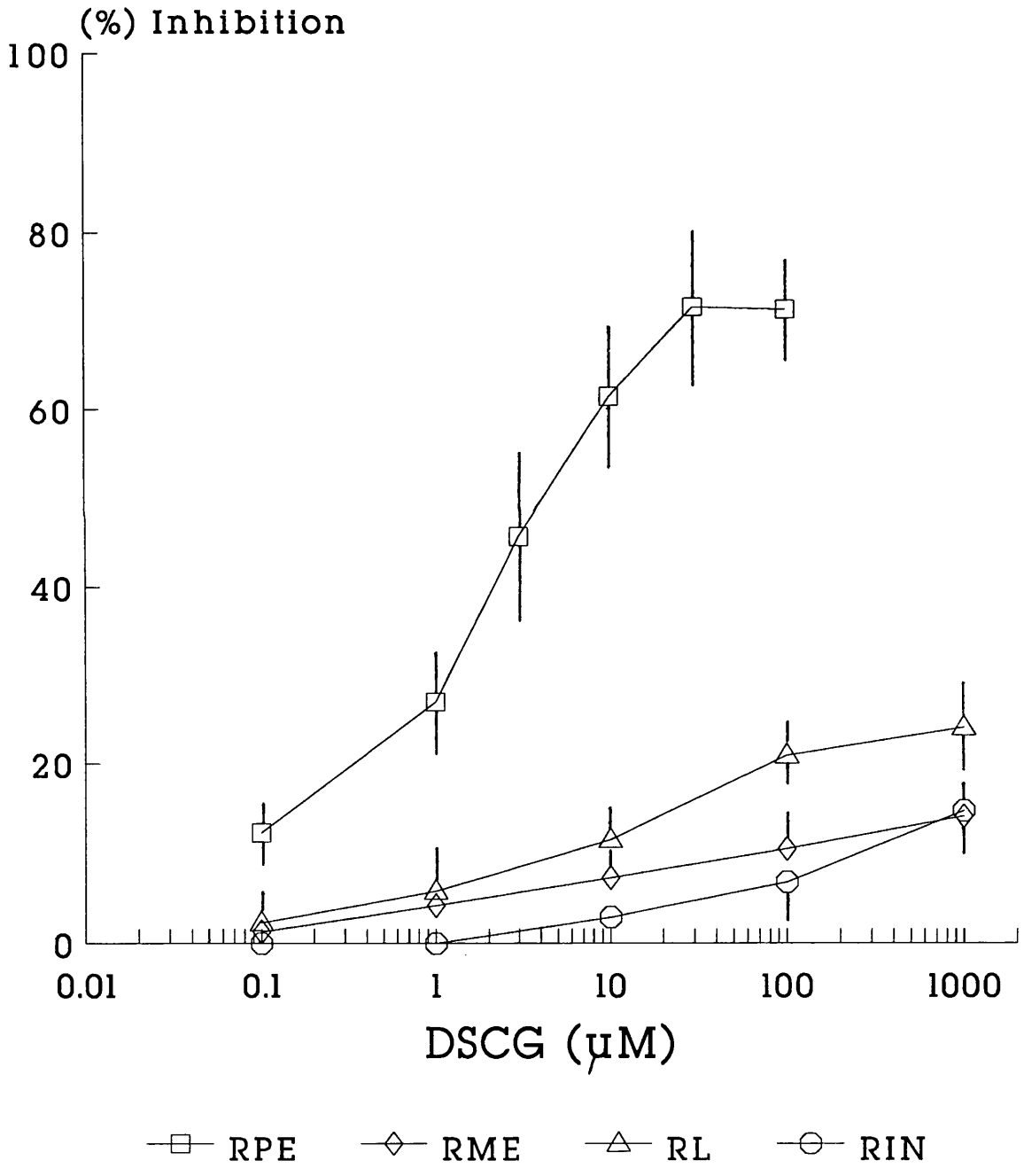


Fig 5.2 Effect of nedocromil sodium on allergen-induced histamine release from actively sensitized rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN). The drug was added simultaneously with stimulus to cells. The control releases were 30.4±6.5 %, n=4, RPE; 30.7±4.1 %, n=5, RME; 22.8±3.6 %, n=5, RL and 9.4±1.6 %, n=5, RIN.

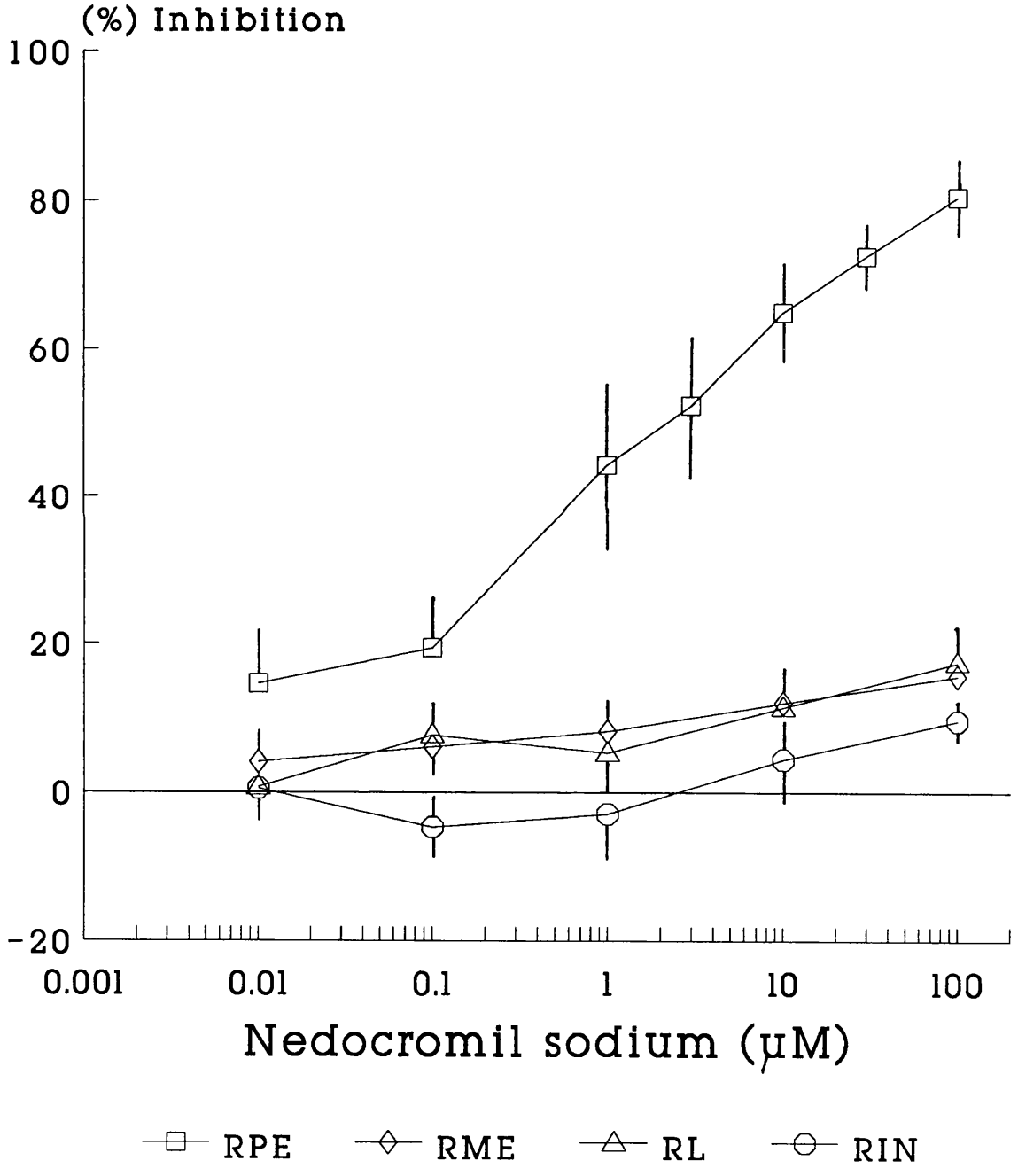


Fig 5.3 Effect of disodium cromoglycate (DSCG) on allergen-induced histamine release from actively sensitized rat peritoneal mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 29.9±5.2 % (0') and 41.1±3.5 % (10') respectively, n=5, paired data.

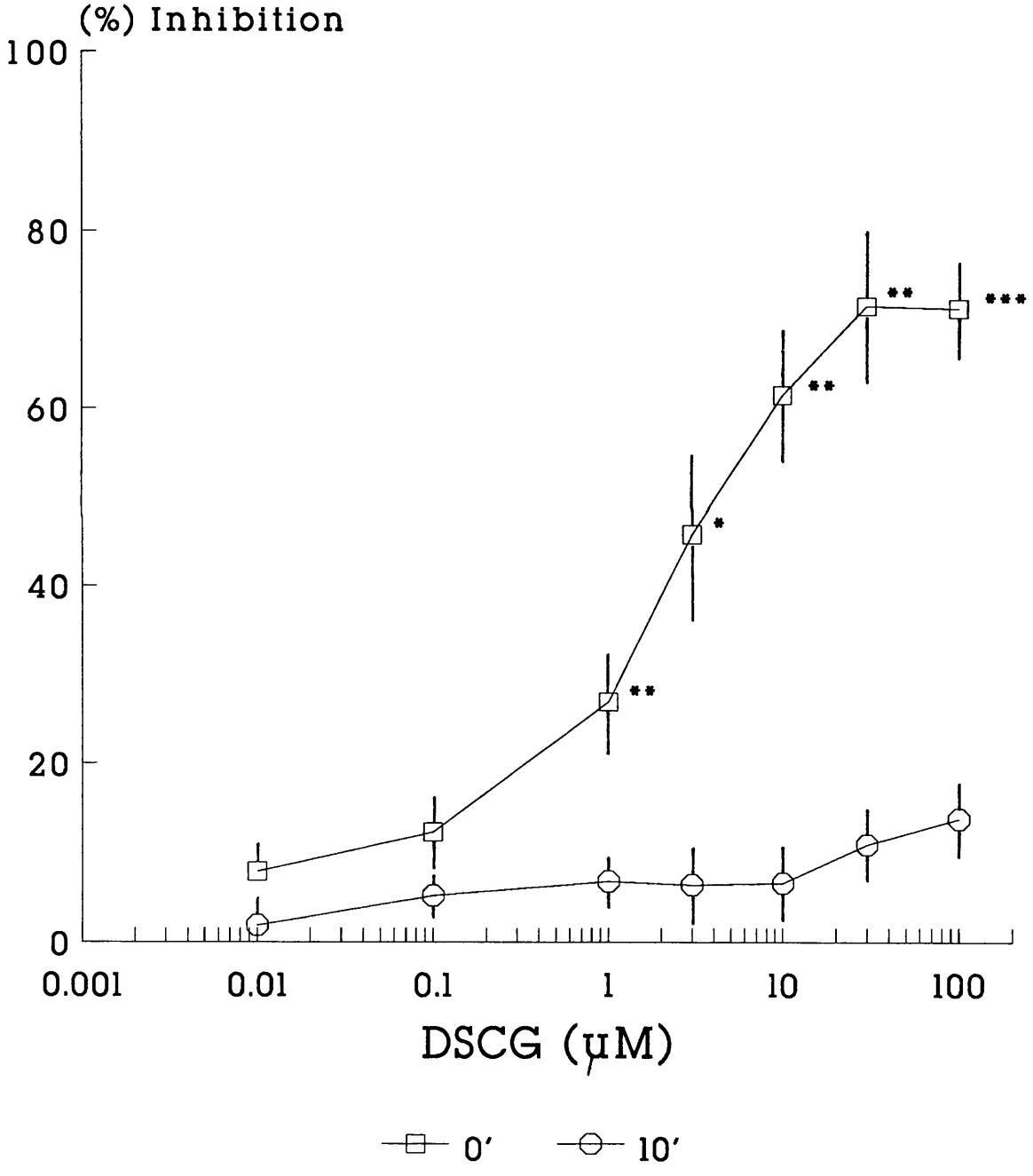


Fig 5.4 Effect of nedocromil sodium on allergen-induced histamine release from actively sensitized rat peritoneal mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 30.4±6.5 % (0') and 36.9±5.0 % (10') respectively, n=4, paired data.

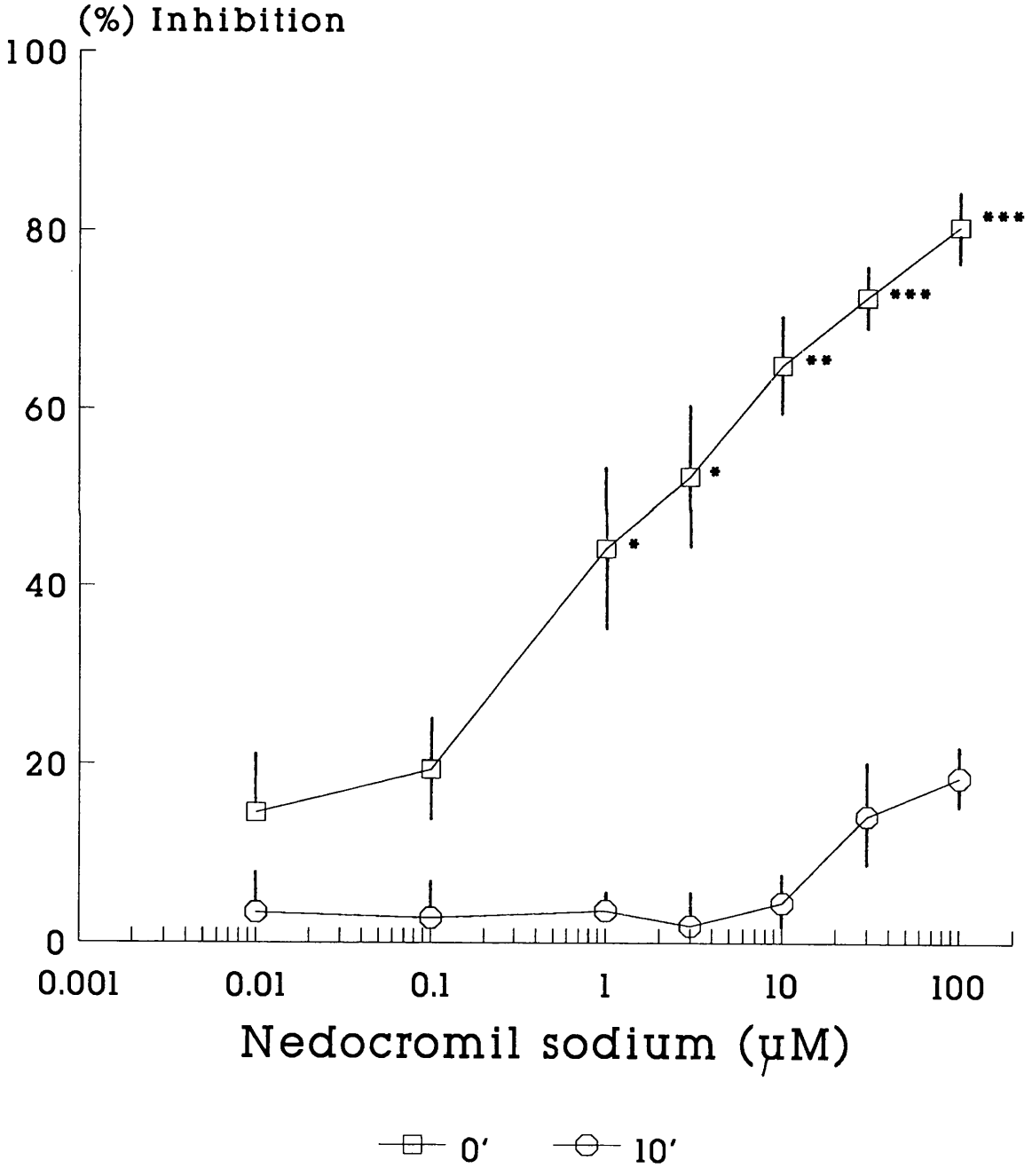


Fig 5.5 Effect of quercetin on allergen-induced histamine release from actively sensitized rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN). The drug was preincubated with cells for 10 min before challenge. The control releases were 44.4±5.1 %, n=5, RPE; 25.7±3.2 %, n=4, RME, 29.1±6.7 %, n=4, RL and 9.6±1.9 %, n=4, RIN.

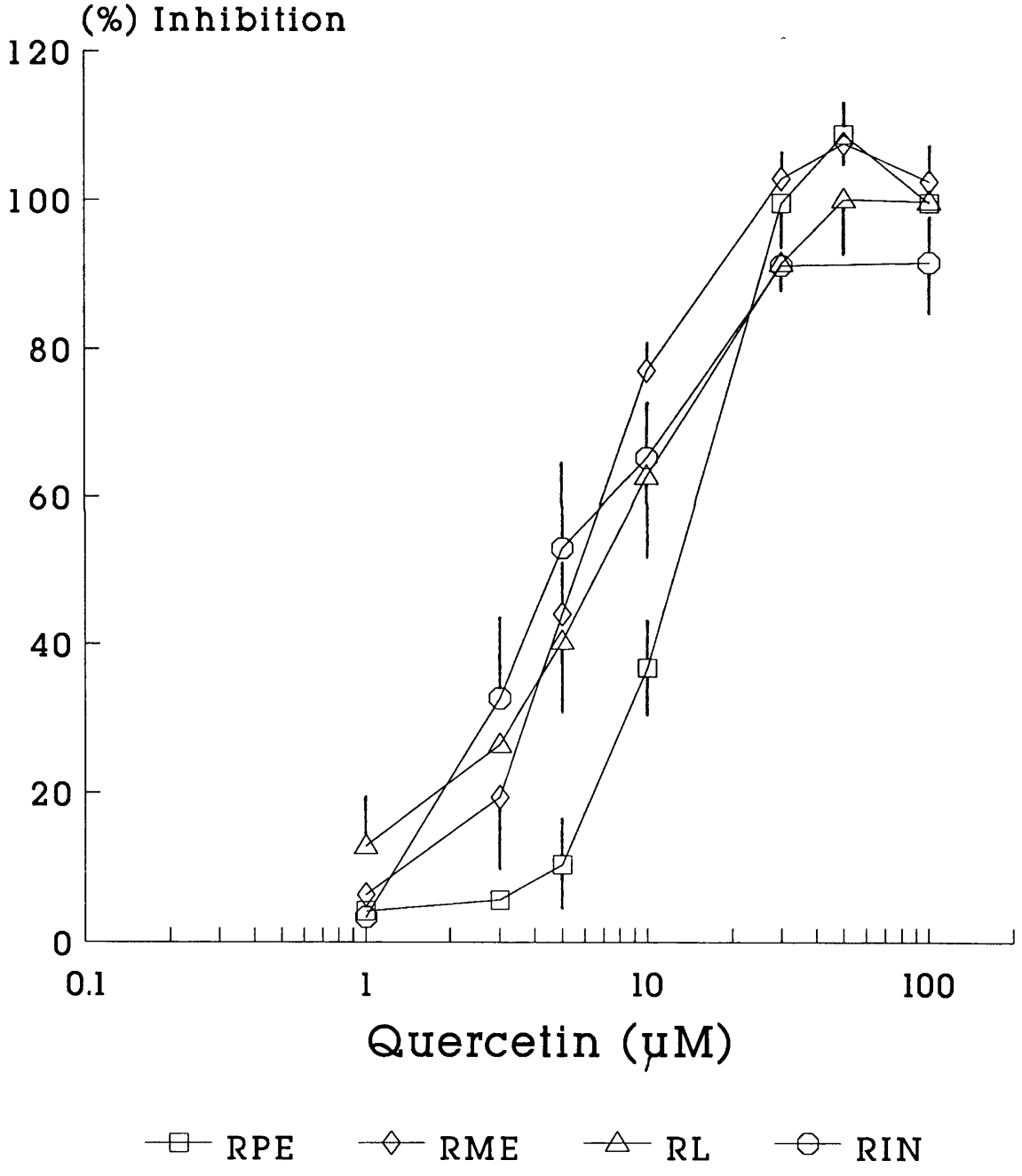


Fig 5.6 Effect of chrysin on allergen-induced histamine release from actively sensitized rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN). The drug was preincubated with cells for 10 min before challenge. The control releases were 54.1±6.6 %, n=4, RPE; 25.7±3.2 %, n=4, RME, 29.1±6.7 %, n=4, RL and 9.0±2.4 %, n=4, RIN.

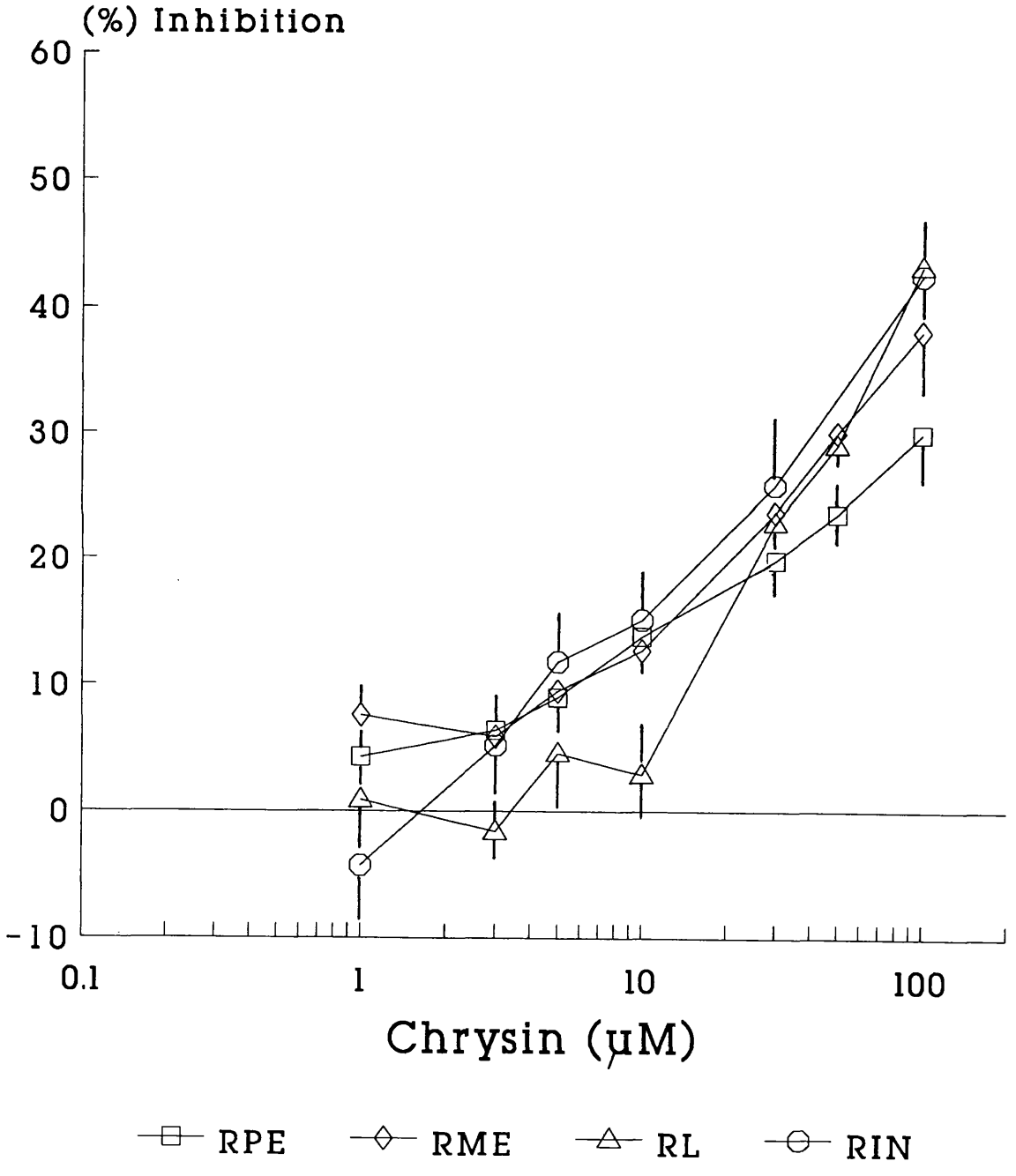


Fig 5.7 Effect of LU 48953 on allergen-induced histamine release from actively sensitized rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN). The drug was added simultaneously with stimulus to cells. The control releases were 22.2±3.2 %, n=4, RPE; 21.1±2.5 %, n=4, RME; 23.7±4.4 %, n=4, RL and 9.7±1.2 %, n=4, RIN.

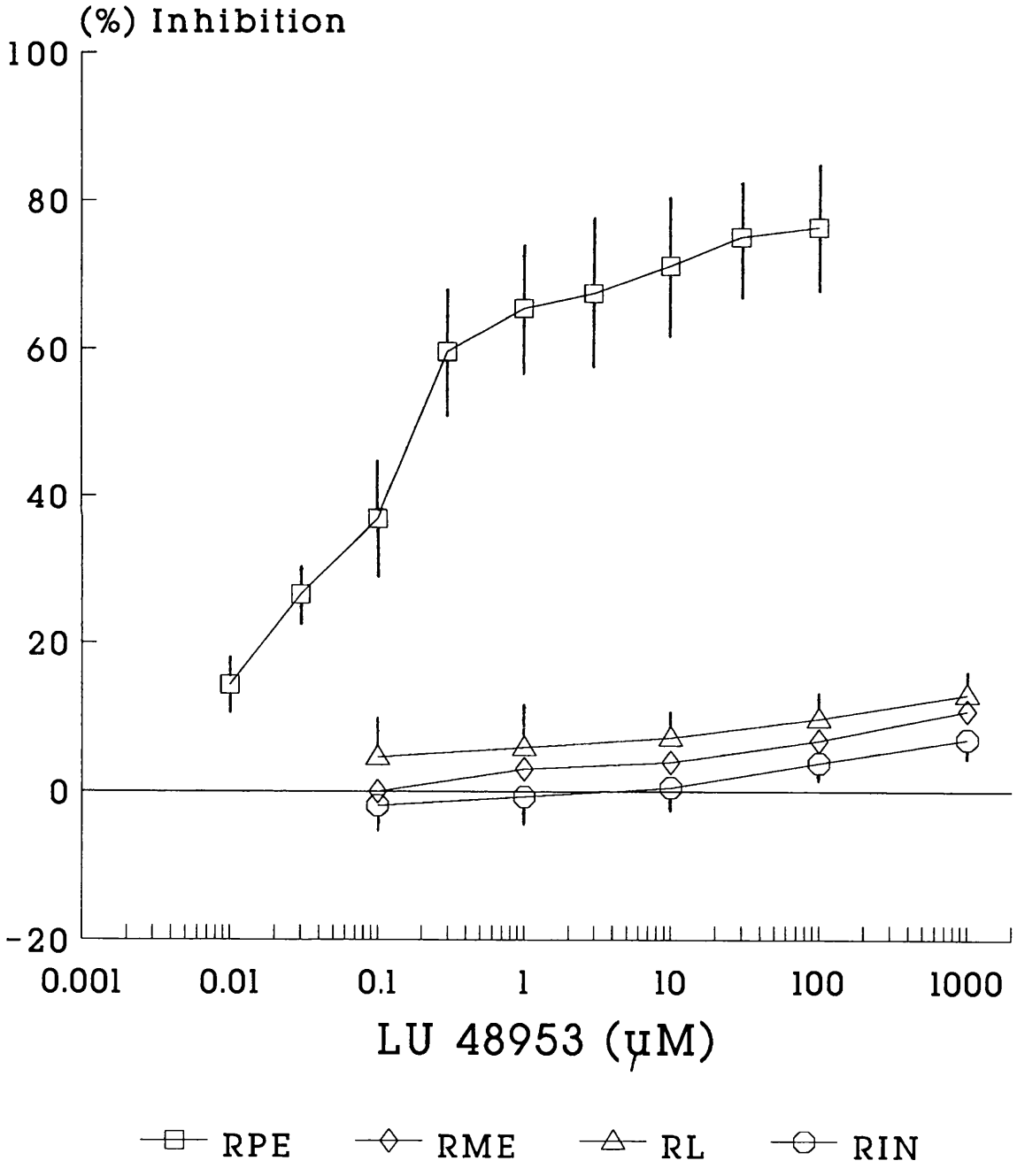


Fig 5.8 Effect of MY 1250 on allergen-induced histamine release from actively sensitized rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN). The drug was added simultaneously with stimulus to cells. The control releases were 30.8±5.6 %, n=4, RPE; 21.1±2.5 %, n=4, RME; 23.7±4.4 %, n=4, RL and 9.7±1.2 %, n=4, RIN.

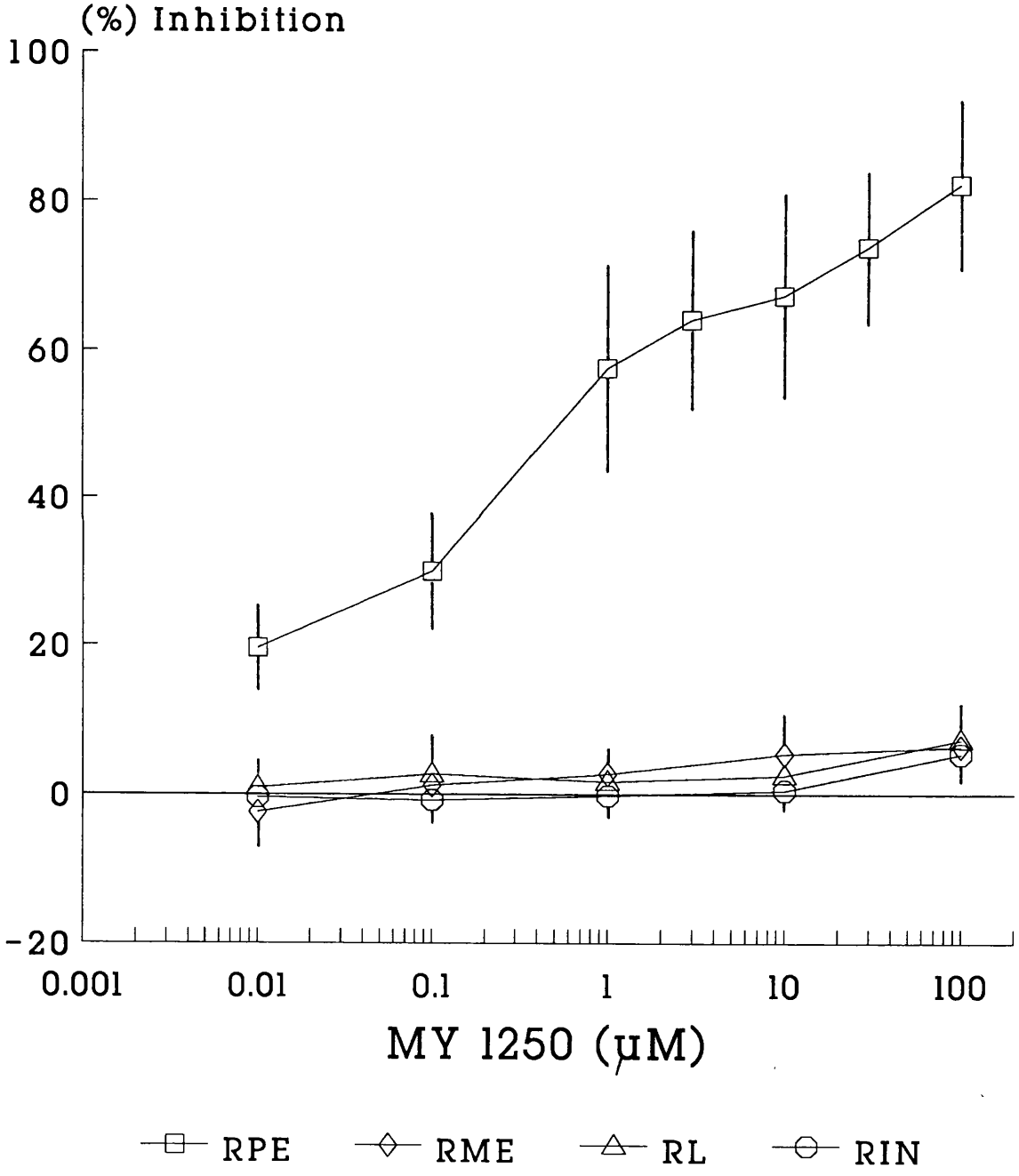


Fig 5.9 Effect of LU 48953 on allergen-induced histamine release from actively sensitized rat peritoneal mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 22.2±3.2 % (0') and 23.0±3.2 % (10') respectively, n=4, paired data.

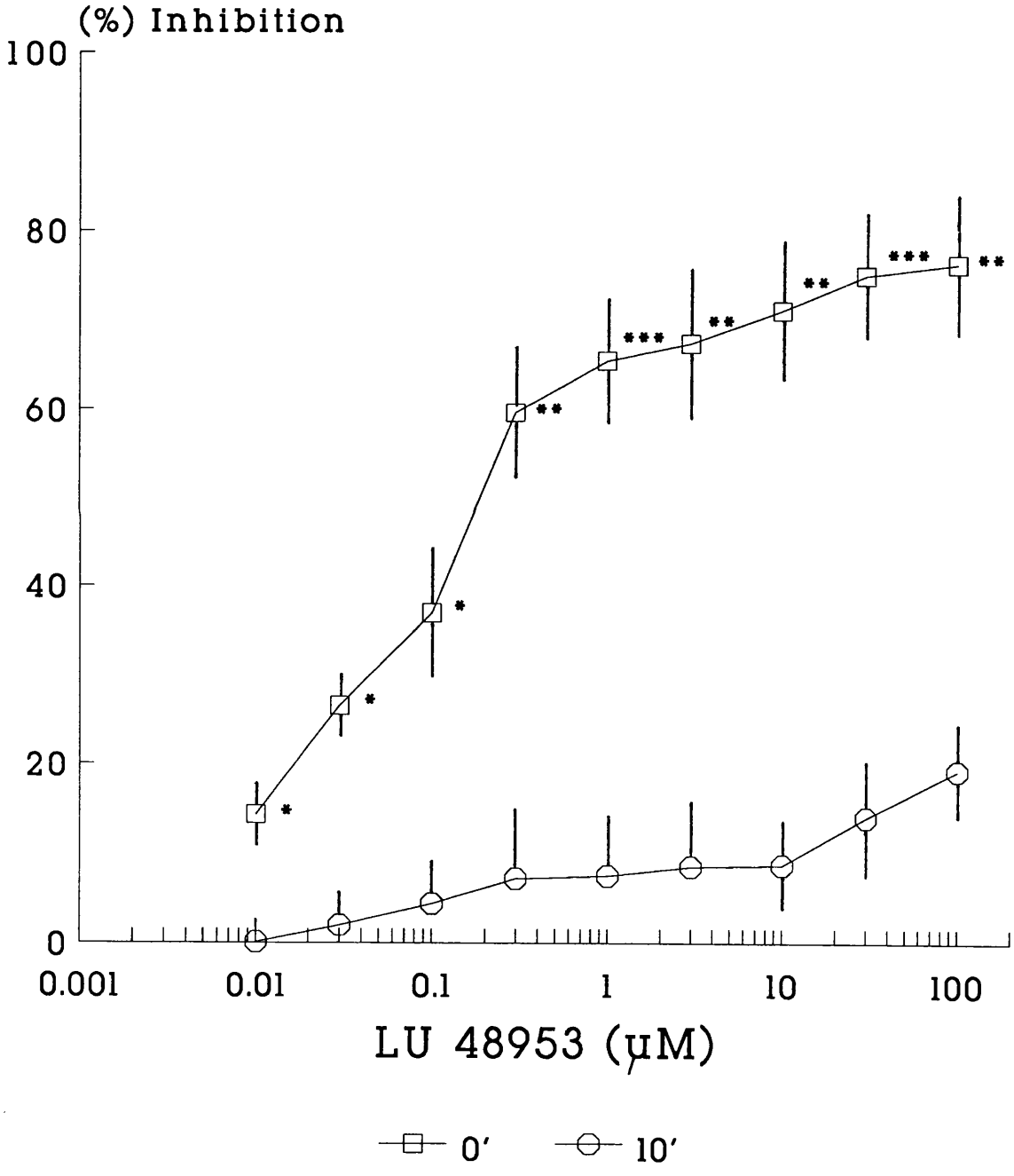


Fig 5.10 Effect of MY 1250 on allergen-induced histamine release from actively sensitized rat peritoneal mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 30.8±5.6 % (0') and 35.3±3.8 % (10') respectively, n=4, paired data.

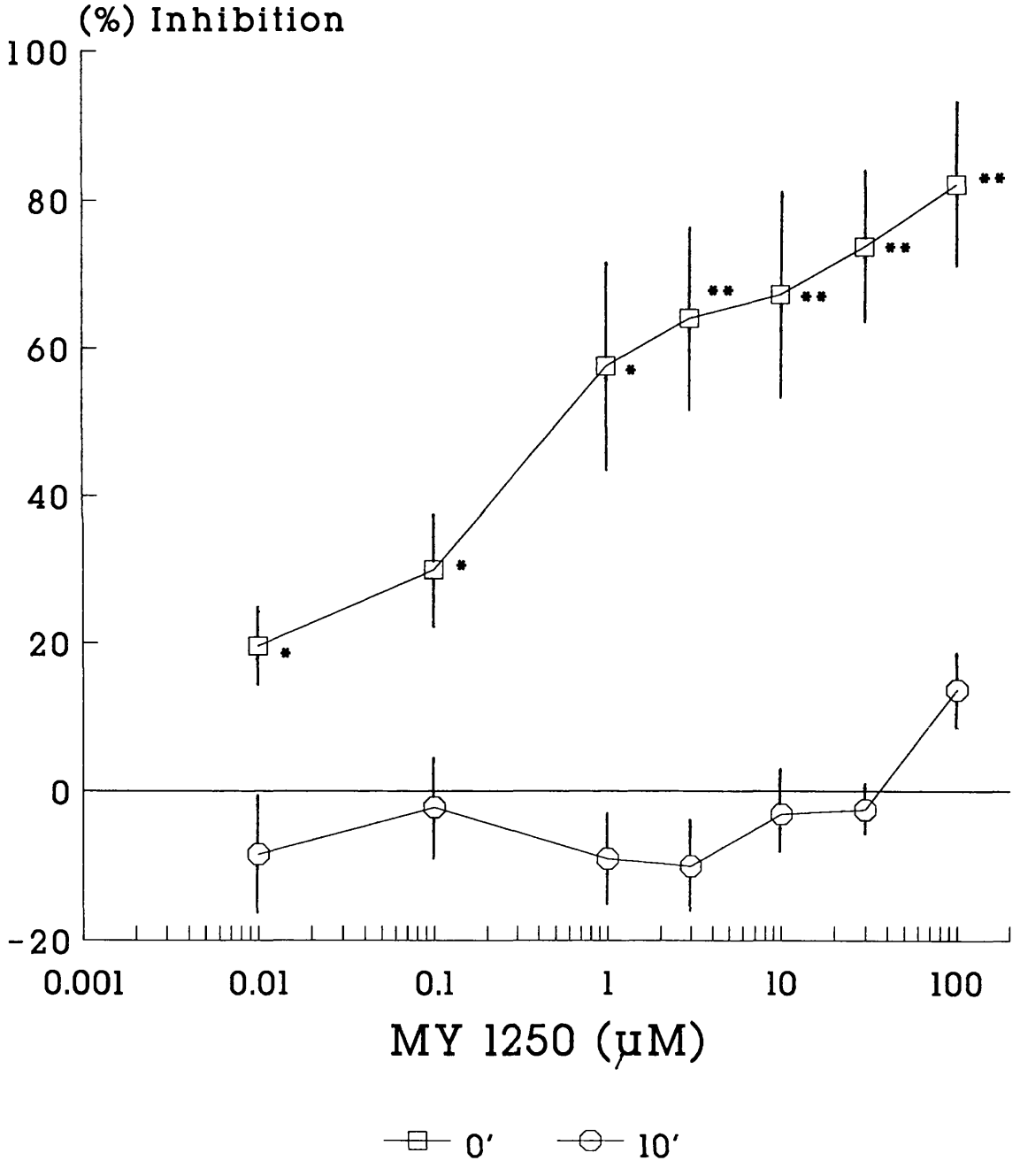


Fig 5.11 Effect of Bu_2cAMP on allergen-induced histamine release from actively sensitized rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN). The drug was preincubated with cells for 30 min before challenge. The control releases were 66.5±1.4 %, n=4, RPE; 44.4±7.2 %, n=4, RME; 23.7±1.4 %, n=4, RL and 10.0±1.3 %, n=4, RIN.

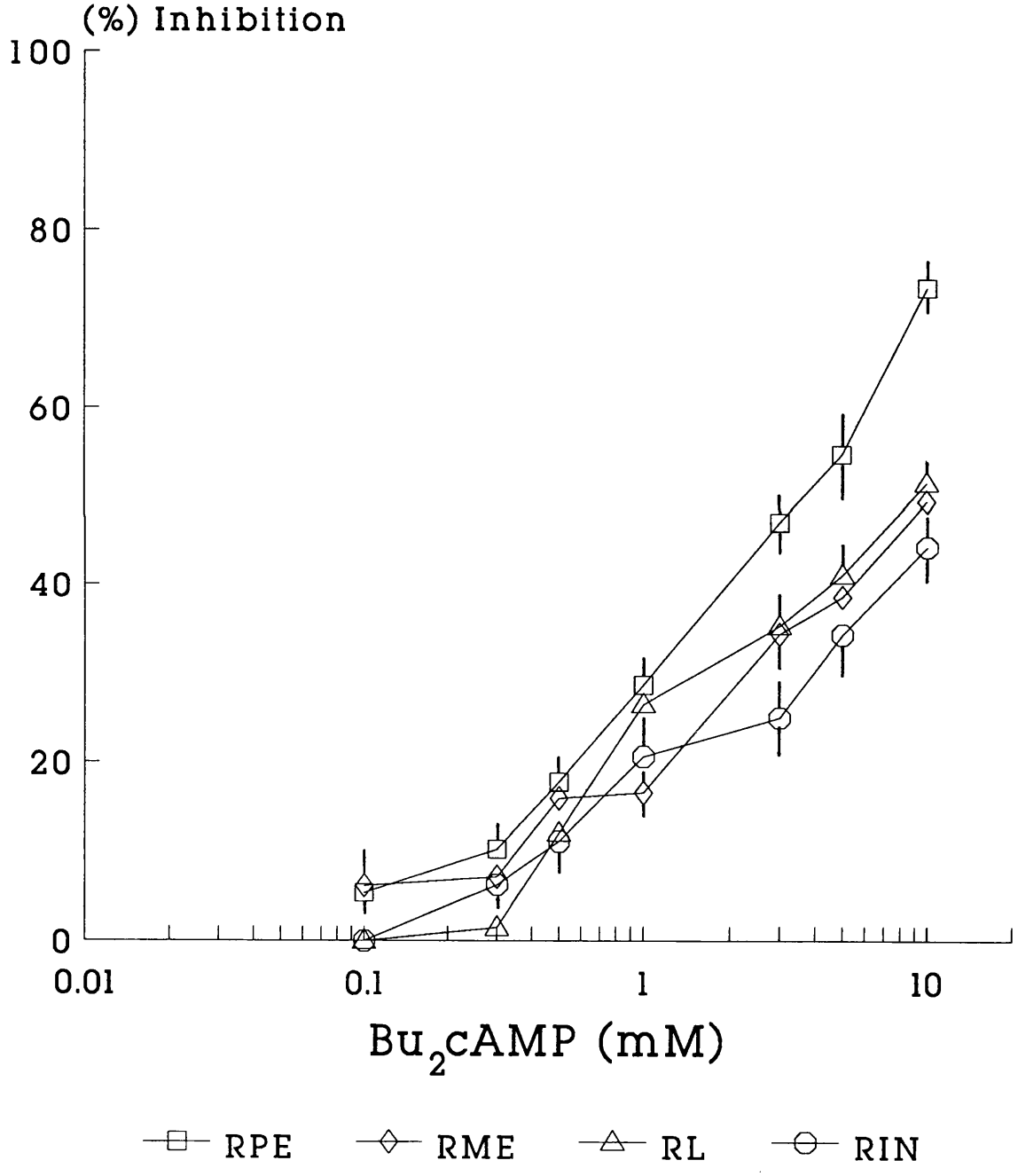


Fig 5.12 Effect of theophylline on allergen-induced histamine release from actively sensitized rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN). The drug was preincubated with cells for 10 min before challenge. The control releases were 42.5±5.2 %, n=4, RPE; 28.8±3.9 %, n=6, RME; 24.6±2.2 %, n=4, RL and 8.4±2.3 %, n=4, RIN.

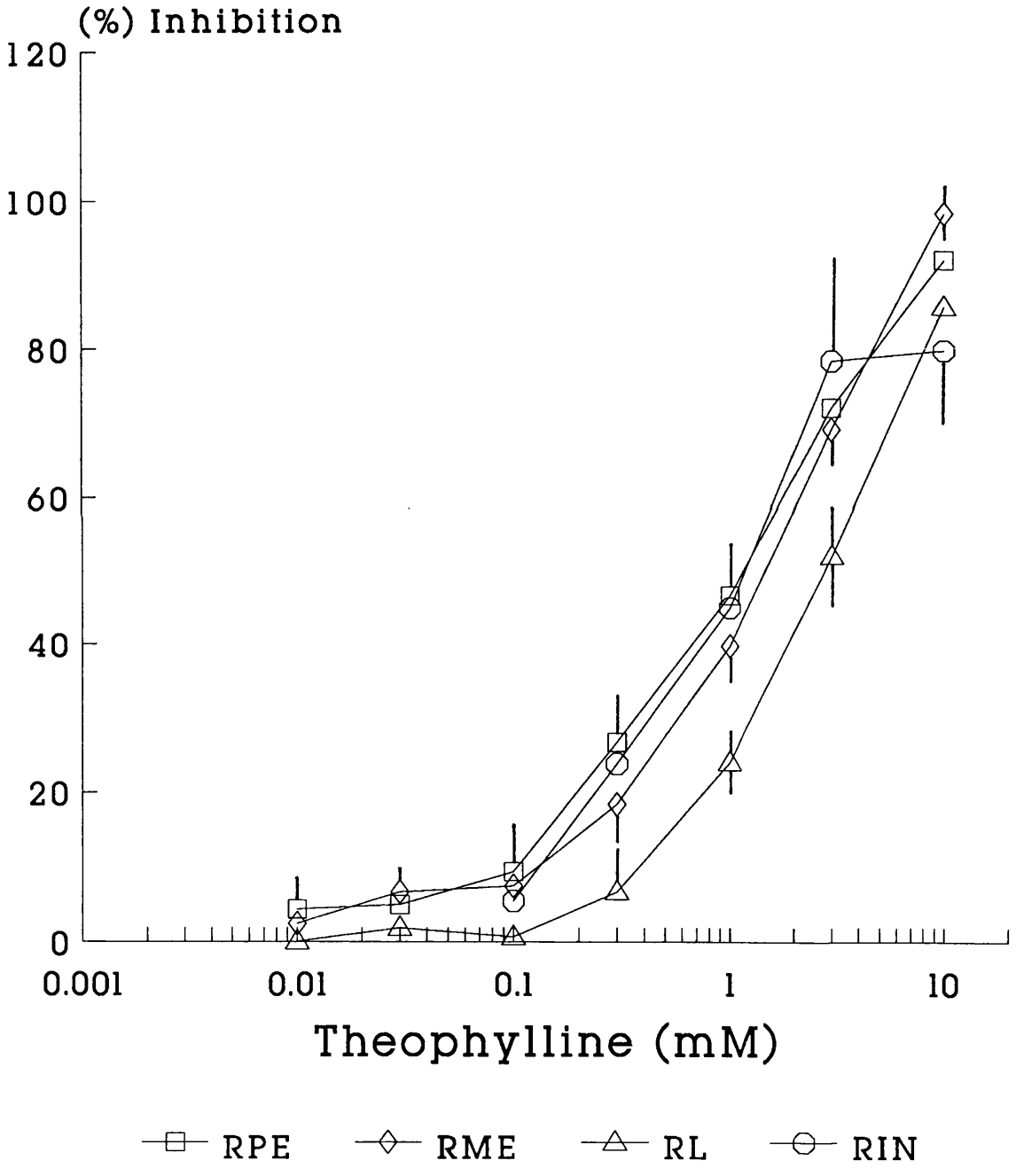


Fig 5.13 Effect of IBMX on allergen-induced histamine release from actively sensitized rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN). The drug was preincubated with cells for 10 min before challenge. The control releases were 43.9±5.2 %, n=6, RPE; 25.7±3.2 %, n=4, RME; 29.0±6.7 %, n=4, RL and 9.0±2.4 %, n=4, RIN.

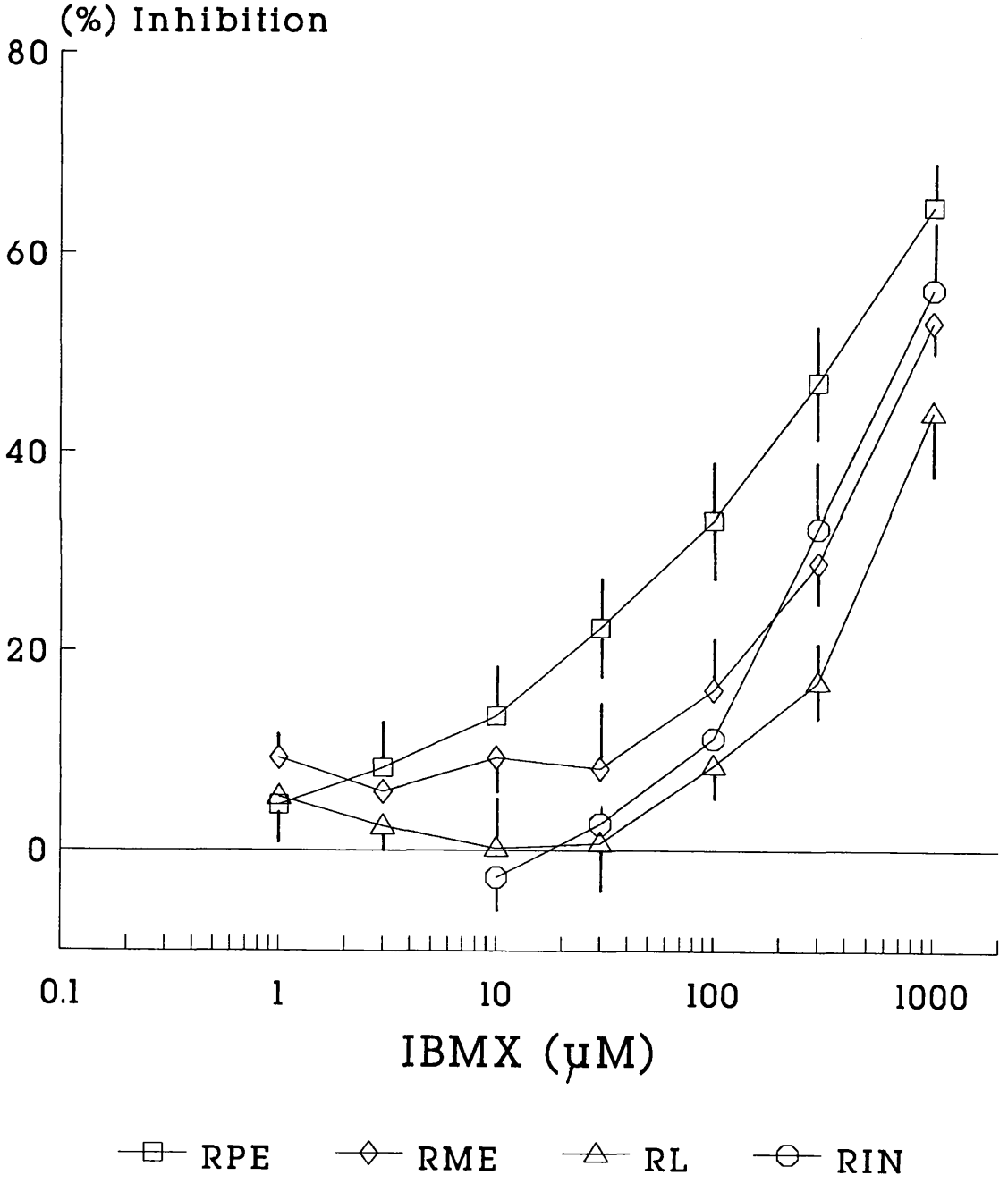


Fig 5.14 Effect of cimetidine on allergen-induced histamine release from actively sensitized rat mast cells from the peritoneum (RPE), mesentery (RME), lung (RL) and intestine (RIN). The drug was preincubated with cells for 10 min before challenge. The control releases were 51.1±5.3 %, n=4, RPE; 39.3±8.3 %, n=4, RME; 24.0±3.0 %, n=4, RL and 10.2±1.7 %, n=4, RIN.

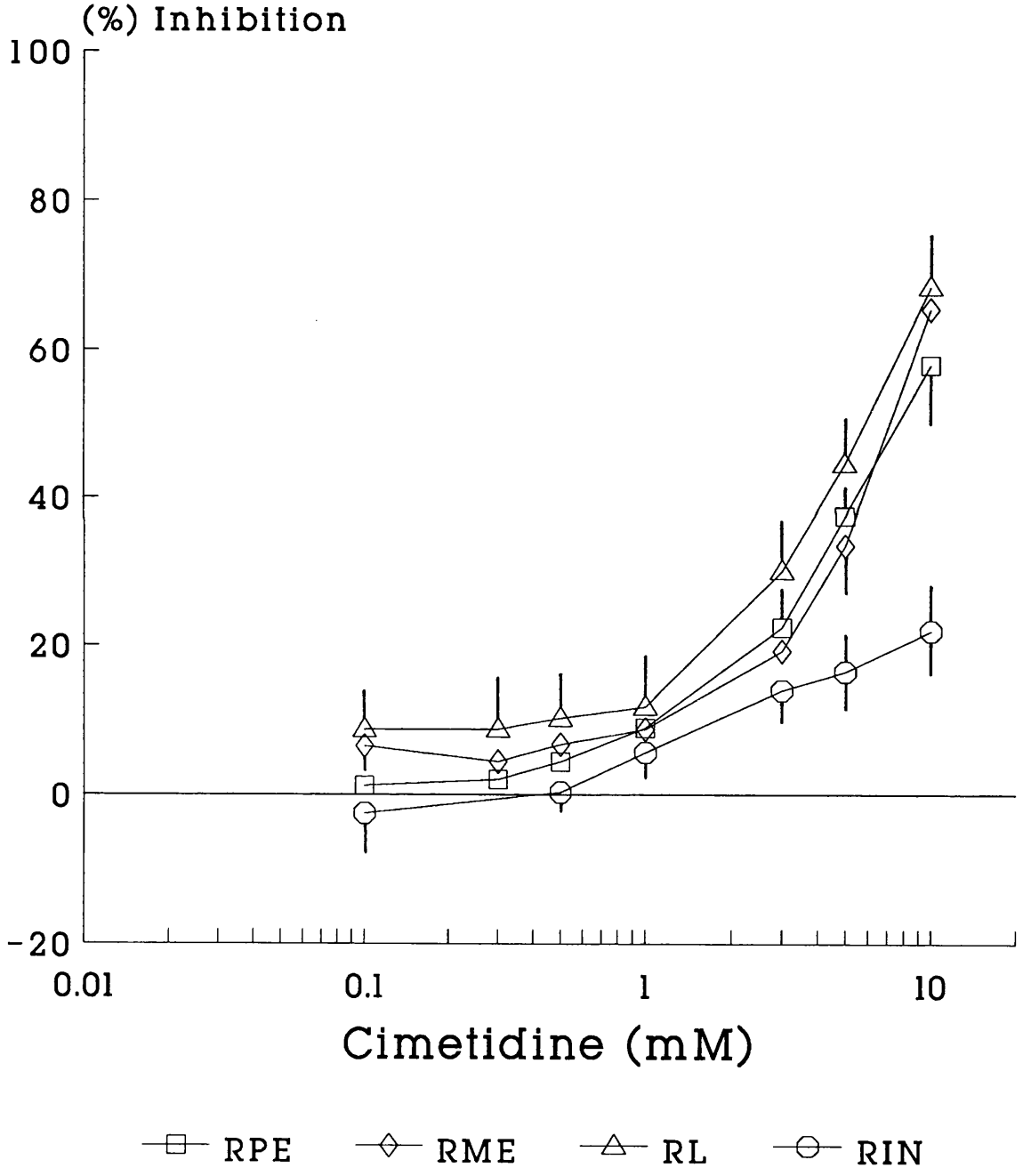


Table 5.1 Effect of DSCG on histamine release from actively sensitized rat mast cells from the mesentery (RME), lung (RL) and intestine (RIN) stimulated with worm allergen.

(a) No preincubation

Concentration (μM)	(%) Inhibition RME n=6	RL n=5	RIN n=5
1000	14.1±3.3	24.0±4.2	14.7±1.9
100	10.5±2.9	20.9±2.5	6.8±3.6
10	7.3±1.8	11.5±2.4	2.9±4.4
1	4.4±2.2	5.8±3.5	-0.3±2.3
0.1	1.3±1.7	2.3±2.2	-1.1±3.2
Control release (%)	30.8±3.3	23.5±3.7	9.4±1.6

(b) 10 min preincubation

Concentration (μM)	(%) Inhibition RME n=6	RL n=5	RIN n=5
1000	12.8±3.1	19.3±2.7	10.4±0.8
100	7.6±2.6	14.5±2.6	4.1±1.3
10	5.9±1.6	10.1±1.7	1.4±1.2
1	2.9±0.8	6.3±1.8	-0.1±0.6
0.1	1.1±1.4	2.5±1.5	-0.6±0.7
Control release (%)	29.4±3.9	24.3±3.1	9.8±1.9

Table 5.2 Effect of nedocromil sodium on histamine release from actively sensitized rat mast cells stimulated with worm allergen.

(a) No preincubation

Concentration (μM)	(%) Inhibition RME n=5	RL n=5	RIN n=5
100	15.6±4.0	17.4±2.8	9.6±0.8
10	12.0±2.8	11.4±4.0	4.4±4.9
1	8.2±2.3	5.3±3.9	-2.9±4.0
0.1	6.1±2.0	7.7±2.4	-4.7±2.4
0.01	4.1±1.9	0.7±2.1	0.5±2.2
Control release (%)	30.7±4.1	22.8±3.6	9.4±1.6

(b) 10 min preincubation

Concentration (μM)	(%) Inhibition RME n=5	RL n=5	RIN n=5
100	9.3±3.4	13.4±2.3	7.3±0.9
10	6.7±2.5	11.2±3.1	3.1±3.1
1	3.8±2.3	6.2±2.5	-1.3±3.5
0.1	2.9±2.3	7.7±3.0	-2.3±1.7
0.01	2.4±0.9	4.3±2.3	-1.8±2.2
Control release (%)	29.1±4.7	24.3±3.1	9.8±1.9

Table 5.3 Effect of LU 48953 on histamine release from actively sensitized rat mast cells stimulated with worm allergen.

(a) No preincubation

Concentration (μM)	(%) Inhibition RME n=4	RL n=4	RIN n=4
1000	10.8±3.7	13.0±1.9	7.0±1.3
100	6.8±2.8	9.7±1.7	3.8±0.8
10	3.9±1.9	7.2±1.5	0.5±1.6
1	3.0±2.6	5.9±3.9	-0.7±2.2
0.1	-0.1±3.8	4.6±3.5	-1.9±1.5
Control release (%)	21.1±2.5	23.7±4.4	9.7±1.2

(b) 10 min preincubation

Concentration (μM)	(%) Inhibition RME n=4	RL n=4	RIN n=4
1000	9.8±3.9	8.6±4.7	9.7±1.0
100	1.7±4.8	5.8±1.2	4.2±1.7
10	2.1±0.9	3.4±0.7	1.9±1.1
1	1.3±2.4	4.1±3.5	1.2±1.5
0.1	3.0±1.8	0.5±0.7	-0.5±1.1
Control release (%)	21.1±3.1	24.6±4.7	9.8±1.2

Table 5.4 Effect of MY 1250 on histamine release from actively sensitized rat mast cells stimulated with worm allergen.

(a) No preincubation

Concentration (μM)	(%) Inhibition RME n=4	RL n=4	RIN n=4
100	6.4±2.3	7.3±3.3	5.4±2.1
10	5.4±3.1	2.5±1.0	0.5±0.5
1	2.7±1.6	1.7±2.0	-0.2±1.1
0.1	1.2±0.9	2.7±3.3	-0.8±1.4
0.01	-2.4±3.1	0.9±1.7	-0.4±1.8
Control release (%)	21.1±2.5	23.7±4.4	9.7±1.2

(b) 10 min preincubation

Concentration (μM)	(%) Inhibition RME n=4	RL n=4	RIN n=4
100	10.5±2.9	11.0±1.8	6.4±1.0
10	4.5±2.8	3.2±1.1	2.6±1.5
1	1.1±2.4	2.8±3.8	-1.3±1.6
0.1	2.4±1.4	1.4±2.0	-1.8±0.4
0.01	2.3±2.2	1.3±2.4	-3.8±0.3
Control release (%)	21.1±3.1	24.6±4.7	9.8±1.2

Table 5.5 Effect of isoprenaline (10 min preincubation) on histamine release from actively sensitized rat mast cells stimulated with worm allergen.

Concentration (μM)	(%) Inhibition RPE n=4	RME n=4	RL n=4	RIN n=4
100	9.8±4.1	7.5±3.3	8.8±4.4	11.6±3.5
30	7.6±4.5	7.4±1.6	6.2±3.7	ND
10	6.6±5.7	6.4±0.8	3.9±4.4	9.0±3.3
3	2.7±1.9	8.7±2.7	5.4±2.8	ND
1	5.0±3.1	5.9±2.3	1.8±2.5	7.7±7.6
0.1	3.4±2.5	8.0±4.1	4.1±1.9	2.8±3.7
0.01	2.0±2.5	6.9±3.0	1.7±2.4	-4.2±1.5
Control release (%)	49.8±7.7	27.0±1.7	30.1±6.5	9.6±2.2

Table 5.6 Effect of salbutamol (10 min preincubation) on histamine release from actively sensitized rat mast cells stimulated with worm allergen.

Concentration (μM)	(%) Inhibition RPE n=4	RME n=4	RL n=4	RIN n=4
100	4.7±2.7	7.4±1.2	4.6±3.7	6.0±6.0
30	3.4±4.1	5.8±2.4	4.6±3.0	ND
10	2.7±3.9	1.8±3.1	2.3±4.4	1.5±4.7
3	2.4±4.2	5.1±2.2	3.0±2.7	ND
1	3.0±3.5	3.0±1.5	0.3±2.4	-6.0±3.1
0.1	1.8±1.1	3.2±2.5	5.1±3.7	-2.3±5.1
0.01	1.1±2.0	4.7±2.5	4.5±4.3	-2.3±5.3
Control release (%)	48.8±7.7	27.0±1.7	30.1±6.5	9.6±2.2

CHAPTER SIX

MAST CELLS FROM HUMAN COLONIC MUCOSA AND SUBMUCOSA/MUSCLE: A COMPARISON WITH HUMAN LUNG MAST CELLS

6.1 Introduction

By virtue of their location, numbers and ability to release a variety of potent inflammatory mediators, the mast cell has long been suggested to play an important role in inflammatory processes of the gastrointestinal tract, and mast cell hyperplasia has been reported to occur in pathological conditions such as coeliac disease [368], inflammatory bowel diseases [28,29] and parasitic infections [369].

The pioneering work of Enerbäck [255,324-326] has revealed the existence of two distinct types of mast cells in the gastrointestinal tract of the rat. Moreover, it has been shown by more recent studies that the same phenomenon may also apply to man. The majority of mast cells in the intestinal mucosa are sensitive to fixation in formaldehyde-based fixatives whereas a large proportion of those cells which reside in the submucosa and muscle are resistant to this treatment [259,260]. A similar differential pattern has been demonstrated with regard to neutral protease content [101,270,271]. The principal mast cell type in the mucosa is one which contains the enzyme tryptase while those found in the submucosa contain two enzymes, tryptase and chymase.

The functional heterogeneity of rat mast cells has already been clearly demonstrated in chapters four and five. Mast cells from the rat intestinal mucosa are relatively non-responsive to a variety of immunological and non-immunological secretagogues as compared to those from other anatomical locations. It is therefore the aim of the present study to investigate whether this phenomenon also extends to man, examining mast cells isolated from the colonic mucosa and submucosa/muscle, and comparing their properties to the more widely studied lung parenchymal mast cells and

blood basophils.

6.2 Methods

All methods used in this study were as described in chapter two.

6.3 Results

6.3.1 Basic properties of human colonic and lung mast cells

Using an identical isolation procedure, free cell suspensions were obtained from both lung and colonic tissues. The enzymic dispersion yielded 0.5±0.1 x 10^6 (lung), 0.9±0.1 x 10^6 (colonic mucosa) and 0.1±0.0 x 10^6 (colonic submucosa/muscle) mast cells per gram wet tissue (n=10) (table 6.1). Mast cells comprised 3.5±0.8, 6.2±1.4 and 6.5±1.3 % (n=10) of the total nucleated cells. The histamine contents per mast cell were 2.6±0.1, 2.7±0.2 and 3.0±0.2 pg (n=10), respectively.

Cells obtained from the three locations were structurally intact as judged under light microscopy, highly viable and exhibited low spontaneous histamine release (table 6.1). Mast cells obtained from the lung parenchyma and colonic mucosa were predominantly sensitive to formaldehyde fixation while about 30 % of those cells in the submucosa/muscle were resistant to this treatment. Finally, mast cells from the three locations were exclusively alcian blue positive and did not counterstain with safranin.

6.3.2 Effects of anti-human IgE

Isolated mast cells from human colonic mucosa and colonic submucosa/muscle released histamine in a dose-dependent fashion in response to stimulation with anti-IgE (fig 6.1). Maximal release for both cell preparations was achieved within 3 min of initial activation (fig 6.2). Moreover, secretion of the amine was totally abolished by preincubation (20 min) of the cells in a glucose-free medium with the combined metabolic inhibitors antimycin A (1 μM) and 2-deoxyglucose (5 mM) (% inhibition,

99.7±1.3 and 101.7±1.8 % respectively, n=4).

In a separate study (n=3), release of newly generated mediators was measured together with the amine. In response to anti-IgE, both colonic mucosal and colonic submucosal/muscle cells released histamine (figs 6.3a and 6.3b), PGD_2 (figs 6.4a and 6.4b) and LTC_4 (figs 6.5a and 6.5b) in a concentration-dependent manner. The production of PGD_2 per 10^6 mast cells, in both cell preparations, was always greater than LTC_4 at all dilutions of anti-IgE employed. As expected, the rate of release of the eicosanoids was much slower than histamine with both PGD_2 (fig 6.6a) and LTC_4 (fig 6.6b) requiring up to 20 min to achieve maximal secretion. Finally, correlation was tested between the release of histamine and the two other mediators and was found to be highly significant ($P < 0.001$) in all cases (figs 6.7-6.9).

Similarly to the two colonic cell preparations, those obtained from the lung parenchyma released histamine in a dose-related fashion upon stimulation with anti-IgE (fig 6.1). The release process was again essentially complete after 3 min (fig 6.2). In contrast, secretion from the basophils was much slower and maximal release was not obtained until 30 min after initial stimulation (fig 6.2).

6.3.3 Effects of anti-human IgG and lectins

Concanavalin A, a lectin which cross-links IgE by binding to sugar moieties in the Fc region, produced a dose-dependent release of histamine from mast cells isolated from the lung parenchyma, colonic mucosa and colonic submucosa/muscle (fig 6.10a). The former cell preparation was the most responsive and the latter the least reactive. In contrast, none of the three cell preparations responded to the lectin from wheat germ, either in the presence or absence of PS (table 6.2). Finally, mast cells from the lung parenchyma and the two colonic portions released histamine in response to anti-human IgG (table 6.3) but the effect observed was much weaker than that produced by anti-IgE.

6.3.4 Effects of calcium ionophores

The calcium ionophore A23187 induced a pronounced secretion of histamine from mast cells isolated from the colonic mucosa and colonic submucosa/muscle (fig 6.10b). In a separate study (n=3), release of newly synthesized mediators was measured simultaneously with the amine. In response to A23187, both colonic mucosal and colonic submucosal/muscle cells released histamine (figs 6.11a and 6.11b), PGD_2 (figs 6.12a and 6.12b) and LTC_4 (figs 6.13a and 6.13b) in a dose-dependent fashion. The production of PGD_2 per 10^6 mast cells, in both cell preparations, was always greater than LTC_4 at all concentrations of A23187 employed. Correlation was tested between the release of histamine and the two eicosanoids and was found to be significant ($P < 0.01$) in all cases (figs 6.14-6.16). Finally mast cells obtained from the lung parenchyma were also highly responsive to the histamine releasing activity of A23187 (fig 6.18).

Similarly to A23187, the calcium ionophores ionomycin (fig 6.17a) and bromo A23187 (fig 6.17b) were potent releasers of histamine from the lung and the two colonic cell preparations. In contrast, the antibiotic chlortetracycline was essentially ineffective against these cells (table 6.4).

6.3.5 Effects of polybasic compounds and neuropeptides

The synthetic polyamine compound 48/80, a potent activator of rat serosal mast cells, was found to be ineffective against mast cells from the lung parenchyma, colonic mucosa and colonic submucosa/muscle (table 6.5). Moreover, isolated human basophils were equally unresponsive. In a separate study (n=3), apart from being an ineffective histamine liberator (table 6.6), this basic agent also failed to induce the production of the eicosanoids PGD_2 (table 6.7) and LTC_4 (table 6.8) by the two colonic cell preparations. This pattern of non-responsivity against the various human histaminocytes was also observed for the other basic agents studied, namely polymyxin (table 6.9), substance P (table 6.10) and $SP_{1\text{-}4}C_{12}$ (table 6.11). The polyamino acids, especially polyarginine, although essentially ineffective as histamine releasers against the lung and colonic mast cells, were capable in inducing the release of this amine from the basophils (tables 6.12 and 6.13).

6.3.6 Effects of other secretagogues

The detergents Triton X-100 (fig 6.18a) was a potent cytotoxic releaser of histamine from lung parenchymal, colonic mucosal and colonic submucosal/muscle mast cells. Another detergent, Tween-20 (fig 6.18b), also demonstrated considerable activity but was more effective against the colonic mucosal cells. The H_2-receptor agonist impromidine (fig 6.19a), a potent histamine releaser on rat peritoneal mast cells, was found to be weakly active against the lung and the two colonic cell preparations. However, in contrast to its activity on the rat cells [chapter 4], the release induced by impromidine from the human cells was cytotoxic since the effect was not blocked by the combined metabolic inhibitors, antimycin A and 2-deoxyglucose.

FMLP, a synthetic bacterial tripeptide, produced a dose-dependent release of histamine from isolated human basophils (fig 6.19b) but had no effect against mast cells from the lung parenchyma and the two colonic portions. Dextran, a clinically used plasma substitute, was ineffective against all human histaminocytes studied, either in the presence or absence of PS (table 6.14).

6.4 Discussion

The present study has demonstrated that functional human mast cells may be obtained by the enzymic dispersion of lung parenchyma, colonic mucosa and colonic submucosa/muscle. Isolated cells from the three locations were highly viable, structurally intact as judged under light microscopy and exhibited a low spontaneous release of histamine. Moreover, these cells have been characterized in terms of their basic histochemical properties and in their functional responses to a number of immunological and non-immunological histamine liberators.

As demonstrated in chapter four, rat mast cells from various anatomical locations contained different amounts of histamine in their secretory granules. Mast cells from the peritoneum contained on average greater than 20 pg of this biogenic amine per cell whereas only about 1 pg was present in those from the intestine. In contrast, and in agreement with other published reports [66,77,250,371], human mast cells cannot be

distinguished by their histamine content since these cells from the lung parenchyma, colonic mucosa and colonic submucosa/muscle all exhibited very similar values.

Rat mast cells may also be distinguished by their sensitivity to formaldehyde fixation and alcian blue-safranin staining. In chapter four, it was shown that the cells isolated from the intestinal mucosa were sensitive to the formaldehyde treatment and stained orthochromatically with alcian blue. In contrast, mast cells from the peritoneum, mesentery and lung were almost exclusively formaldehyde resistant and stained metachromatically with safranin. The present study has also demonstrated the existence of two types of human mast cells based on their formaldehyde sensitivity; those isolated from the lung parenchyma and colonic mucosa were predominantly susceptible to formaldehyde fixation, in contrast to the colonic submucosa/muscle which contained about 30 % formaldehyde resistant mast cells. This figure is somewhat smaller than that published by Rees et al [371] who reported greater than 90 % formaldehyde sensitive mast cells in the colonic muscle. The reasons for this discrepancy are not obvious, but may reflect the fixation time employed (24 hr as compared to 2 hr) or the basic dye used for staining (alcian blue as compared to toluidine blue).

The above results indicate that the majority of mast cells in human lung and colon have histochemical properties similar to the rat intestinal mucosal mast cells. Indeed, in keeping with this theme, these human cells can be stained by alcian blue but cannot be counterstained with safranin.

Cross-linkage of cell-surface IgE by anti-IgE and concanavalin A stimulated a concentration-related release of histamine from mast cells isolated from the human lung parenchyma, colonic mucosa and colonic submucosa/muscle. In each case, the lung cells were the most responsive and the submucosal/muscle cells the least reactive. The mechanisms underlying this difference in responsivity are unknown, but may relate to IgE receptor density on the cell types, a phenomenon which has been clearly demonstrated in rat mast cells [333,334]. However, despite this slight difference in concentration-response characteristics, these isolated human mast cells exhibited similarities in the kinetics of anti-IgE-induced histamine release, requiring about 3 minutes to achieve maximal secretion. In contrast, release of this amine was somewhat

slower in the blood basophil, needing about 30 minutes to complete the process.

In addition to the secretion of histamine, the newly generated mediators PGD_2 and LTC_4 were also dose-dependently released from the two colonic cell preparations upon stimulation with anti-IgE. However, the rate of release of the two eicosanoids was much slower than histamine, requiring 20 minutes to achieve optimal secretion. The production of the prostanoid per 10^6 mast cells, in each case, was always greater than LTC_4 at all dilutions of anti-IgE employed. However, it has been demonstrated by Fox et al [77] that the major sulphidopeptide leukotriene observed in supernatants from activated human intestinal mucosal mast cells is LTE_4. This finding is in contrast to human lung mast cells, in which the major sulphidopeptide leukotriene is LTC_4 [77]. The above authors went on to suggest that considerable degradation of sulphidopeptide leukotrienes occurs as these products are released from preparations of human intestinal mast cells, apparently by proteases and other degradative enzymes in these preparations. With this in mind, and with the knowledge that the radioimmunoassay employed in the present study is about 2-fold less sensitive for LTE_4 as compared to LTC_4, the true quantity of LTC_4 released from mast cells from the two colonic portions may be considerably greater than the observed values.

Capron et al [372] proposed that anti-IgE has the ability to cross-link cell surface bound IgE via two classes of receptors; the FcR_1 receptor which is present on mast cells and the FcR_2 subtype on macrophages, eosinophils and platelets. With this in mind, it is therefore possible that these cell types may play a role in the release of eicosanoids from the two colonic cell preparations. The contributing factor, however, if any, is likely to be small since the FcR_1 receptor has a much higher affinity for IgE than the FcR_2 receptor [372]. Moreover, spontaneous release of PGD_2/LTC_4 as measured in assay controls was low and correlation between the release of histamine and the two eicoasnoids was found to be highly significant ($P < 0.001$) in all cases. Finally, it is now well documented in the literature that purified mast cells from the human lung parenchyma are also capable of synthesizing and releasing both PGD_2 and LTC_4 upon immunological activation [118,276,339,350,373].

There is now considerable evidence to suggest that anaphylactic antibodies of

the IgG_4 subclass can act synergistically with IgE antibodies [374,375]. Gwynn et al [376] observed that in patients suffering from allergic conditions that included asthma and atopic eczema, raised serum levels of IgE were frequently accompanied by raised levels of IgG_4. In a more recent study, Nolte et al [377] reported that enzymically isolated intestinal mucosal mast cells from patients with inflammatory conditions of the intestine released significant amounts of histamine, in a dose-dependent fashion, on stimulation with anti-IgG_4. In contrast, mast cells from healthy controls were unresponsive to anti-IgG_4 challenge. Moreover, polyclonal anti-IgG was unable to activate mast cells from both groups of patients. These results indicate a possible pathophysiological role of IgG_4 as a reaginic antibody in human allergic and inflammatory processes. The results of the present study were in slight contrast to those obtained by Nolte et al [377] since isolated mast cells from normal human lung parenchyma, colonic mucosa and colonic submucosa/muscle released low but consistent levels of histamine upon challenge with whole anti-IgG serum.

In common with anti-IgE, the calcium ionophore A23187 induced the parallel release of histamine, PGD_2 and LTC_4 from the two colonic cell preparations in a dose-dependent fashion. In each case, the production of the prostanoid per 10^6 mast cells was again greater than LTC_4 at all concentrations of ionophore tested. However, the observed release of the eicosanoids may not be as closely attributed to mast cells as with anti-IgE, since A23187 has the potential to cause calcium-dependent secretion of these lipid mediators from many other cell types [125]. Despite this, the present study has demonstrated good correlation ($P < 0.01$ in all cases), for both colonic cell preparations, between the release of histamine and the two newly generated eicosanoids.

Isolated mast cells from the two colonic portions, along with those from the lung parenchyma, also released histamine upon stimulation with two other calcium ionophores, namely bromo A23187 and ionomycin. These results therefore suggest that, in each case, an elevated level of the cation in the cell cytosol is a necessary and sufficient trigger for secretion [19,136,149]. In contrast, the antibiotic chlortetracycline, which has been shown to exhibit ionophoretic properties [378], was essentially ineffective as a histamine releaser against these human mast cells. A more recent unpublished study by Mustafa et al (personal communication) has questioned the

ability of chlortetracycline to act as a calcium ionophore. They found that in the presence of both calcium and lanthanum ions in the extracellular medium, histamine release from rat peritoneal mast cells induced by chlortetracycline was inhibited in a dose-dependent fashion. From this, they proposed that chlortetracycline binds to specific sites on the surface of the mast cell, and this causes the opening of calcium channels in the cell membrane. Influx of calcium then follows, ultimately resulting in histamine release. With this in mind, it is possible that the non-responsiveness of human mast cells to chlortetracycline may be due to the absence of these binding sites.

The inability of the synthetic polyamine compound 48/80 to activate human lung parenchymal mast cells and blood basophils has been clearly demonstrated in chapter three. In the present study, in addition to its ineffectiveness as a histamine releaser, this polybasic agent also failed to release the newly generated eicosanoids PGD_2 and LTC_4 from mast cells isolated from the human colonic mucosa and colonic submucosa/muscle. Moreover, the other basic agents studied, namely polymyxin, substance P, $SP_{1\text{-}4}C_{12}$, polylysine and polyarginine were very weak or non-releasers of histamine from these colonic mast cells. These results have therefore added further weight against a generalized model of neurogenic inflammation involving mast cell-peptide interactions in man; the skin mast cell now seems to be the only cell type that possibly fits this hypothesis.

The detergents Triton X-100 and Tween-20 are members of a group of mast cell secretagogues collectively known as non-selective releasers [53]. These agents are cytotoxic and exert their actions by disrupting the mast cell membrane, thereby liberating all of the intracellular contents including histamine. In keeping with this mechanism, Triton X-100 was a potent releaser of histamine from all three human mast cell preparations. Tween-20, in contrast, was most active against the colonic mucosal cells, suggesting that there may be possible structural differences in the cell membranes between the various mast cell populations. Finally, it is interesting to note that in contrast to its effect on rat mast cells [chapter four], histamine release induced by the H_2-receptor agonist impromidine from human mast cells, was observed to be cytotoxic.

The possible role of FMLP and FMLP-related peptides in the defence against bacterial infection has already been discussed in chapter four. In the present study, and in agreement with the results of Siraganian and Hook [349], FMLP induced a dose-dependent release of histamine from human basophils but had no effect against mast cells isolated from the lung parenchyma, colonic mucosa and colonic submucosa/muscle. However, it has been reported [277,280] that a slight release was observed at high concentrations with human cutaneous mast cells. The physiological significance of this effect, if real, is not immediately clear, but it may reflect the developmental response of the skin cells to their local microenvironment, and in particular their close association with blood vessels, whereby they have acquired a role in host resistance in addition to immediate hypersensitivity reactions.

The polysaccharide dextran has been employed clinically as a plasma substitute and blood volume expander. Adverse reactions to dextran have been observed in man but the extent to which they involve mediator release is controversial. The present study has demonstrated that dextran is an ineffective releaser of histamine from both human lung and colonic mast cells, as well as from human basophils. These data therefore support the theory that the systemic reaction induced by dextran in man is not mast cell or basophil-mediated, or that it is a genuine IgE-mediated anaphylactic response in a subgroup of sensitized patients.

The present investigation clearly demonstrates that in contrast to the situation in the rat, enzymically isolated human mast cells from the lung parenchyma, colonic mucosa and colonic submucosa/muscle are, in many respects, functionally similar in their responses to a number of immunological and non-immunological secretagogues. Some slight histochemical differences, however, were observed, notably in their sensitivities toward formaldehyde fixation.

Fig 6.1 Histamine release induced by anti-human IgE from isolated human mast cells from the lung (HL), colonic mucosa (HCM), colonic submucosa/muscle (HCS), and human basophils (HB) (n=7-10).

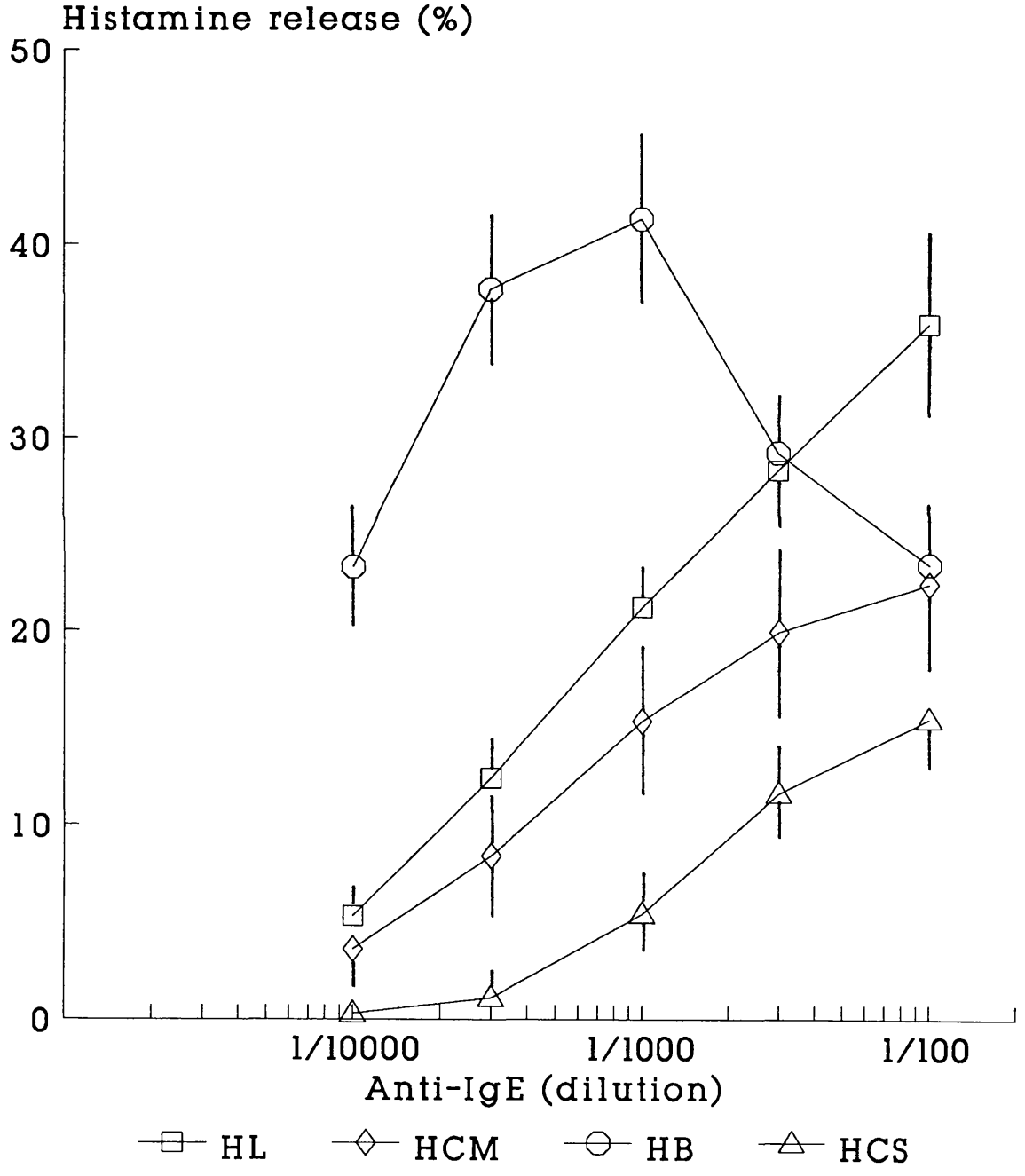


Fig 6.2 Kinetics of histamine release induced by anti-human IgE from isolated human mast cells from the lung (HL), colonic mucosa (HCM), colonic submucosa/muscle (HCS), and human basophils (HB) (n=3-4).

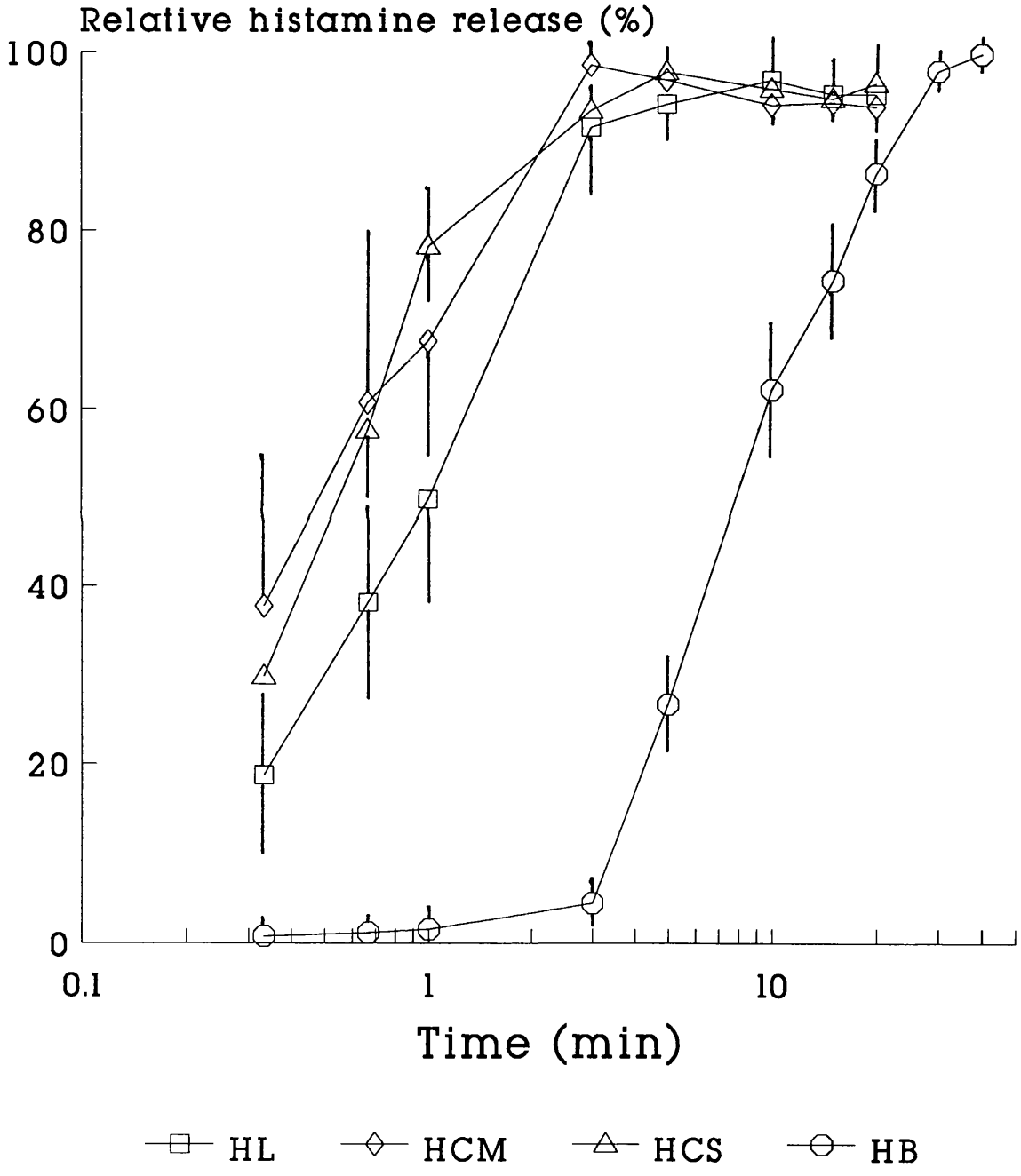


Fig 6.3 Histamine release induced by anti-human IgE from isolated human mast cells from (a) colonic mucosa (n=3) and (b) colonic submucosa/muscle (n=3).

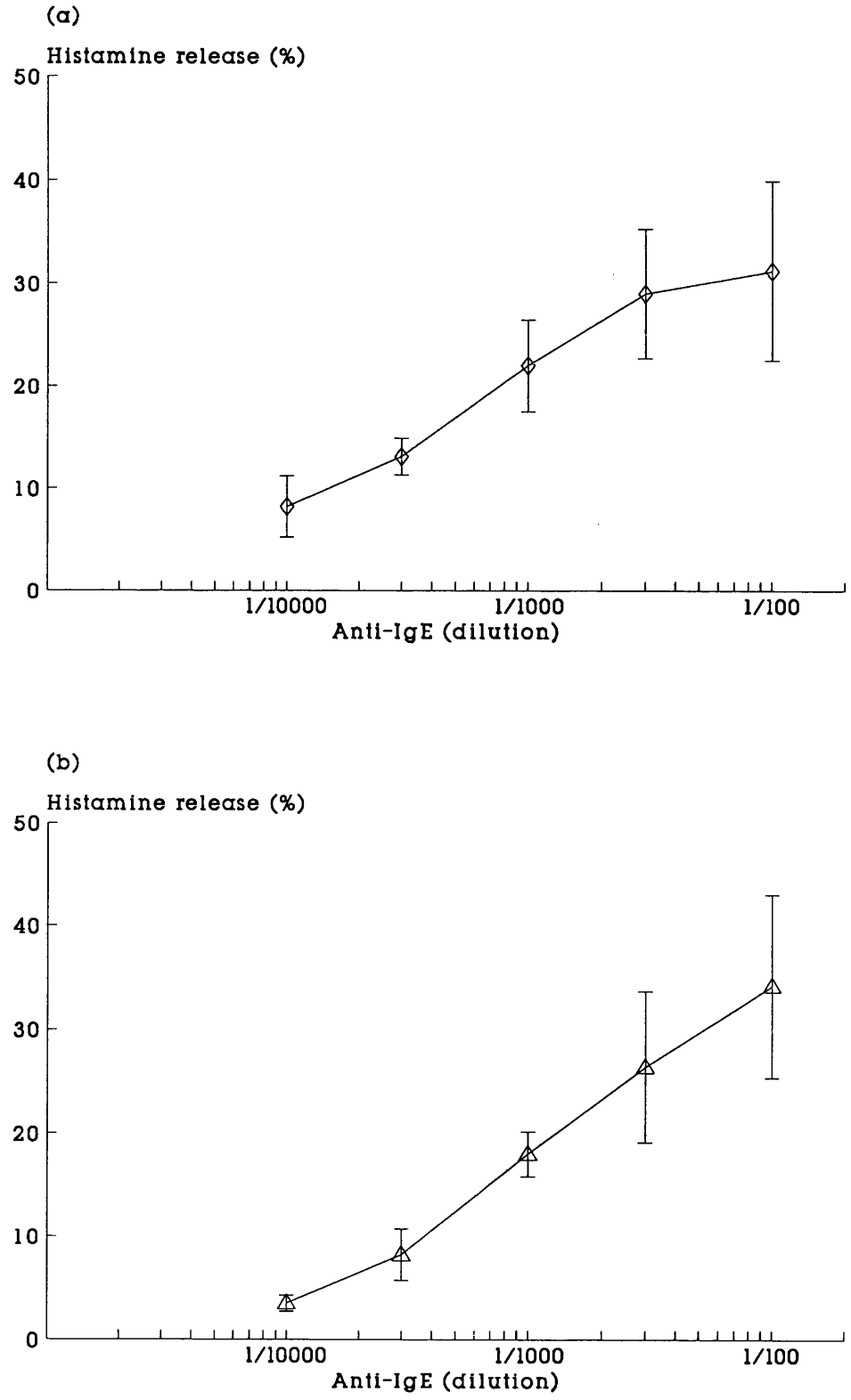


Fig 6.4 PGD_2 release induced by anti-human IgE from isolated human mast cells from (a) colonic mucosa (n=3) and (b) colonic submucosa/muscle (n=3).

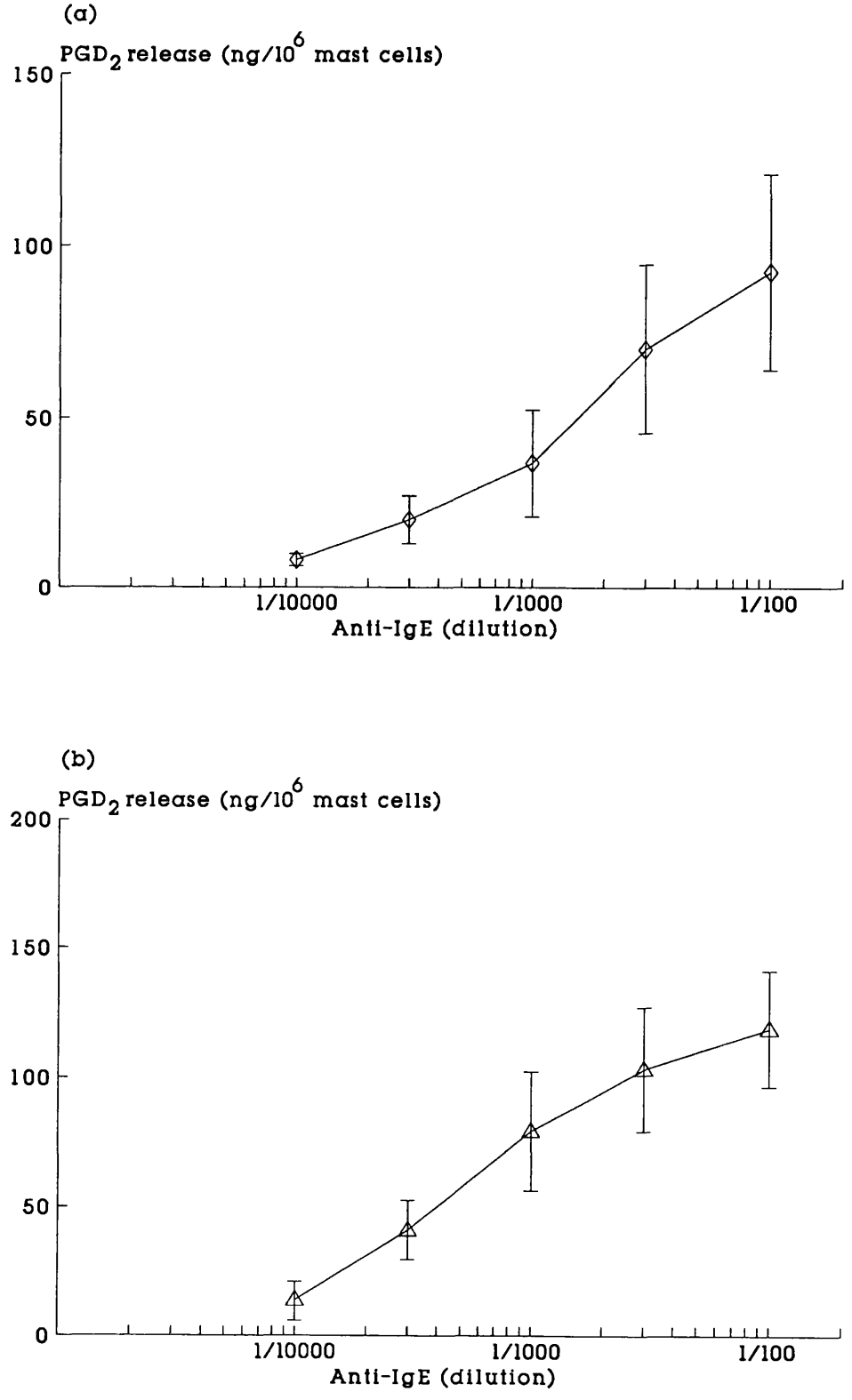
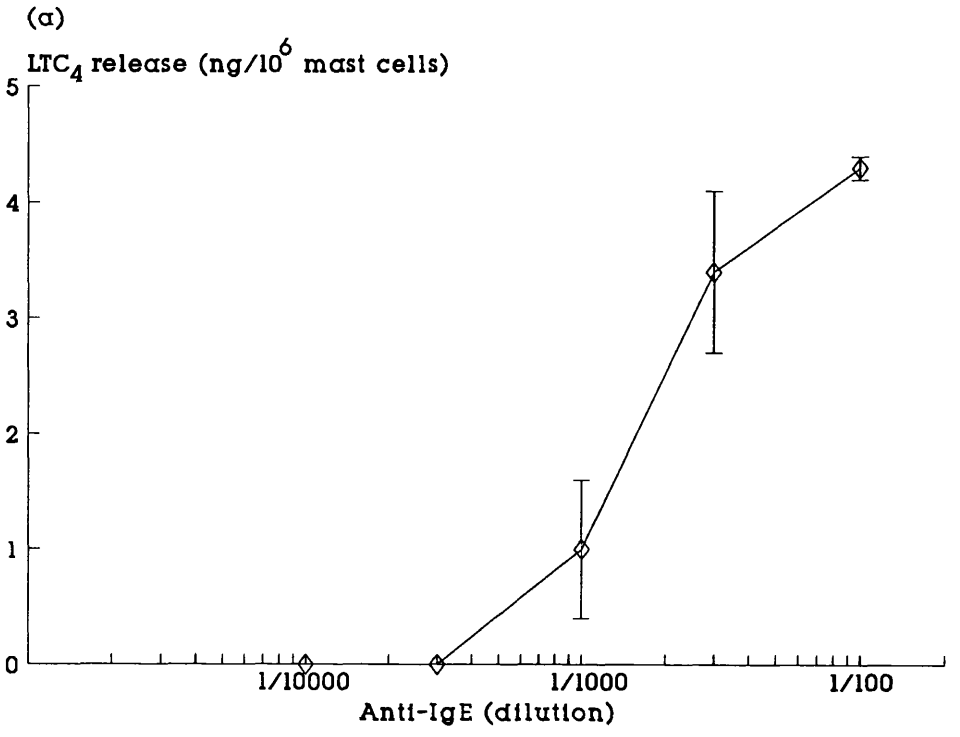


Fig 6.5 LTC_4 release induced by anti-human IgE from isolated human mast cells from (a) colonic mucosa (n=3) and (b) colonic submucosa/muscle (n=3).



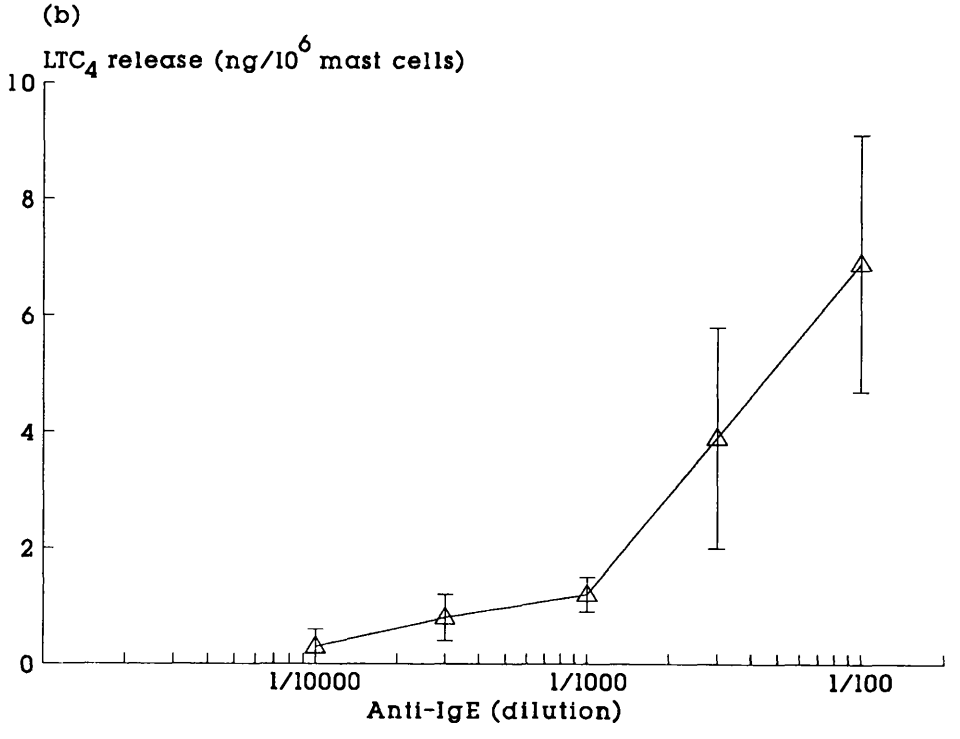
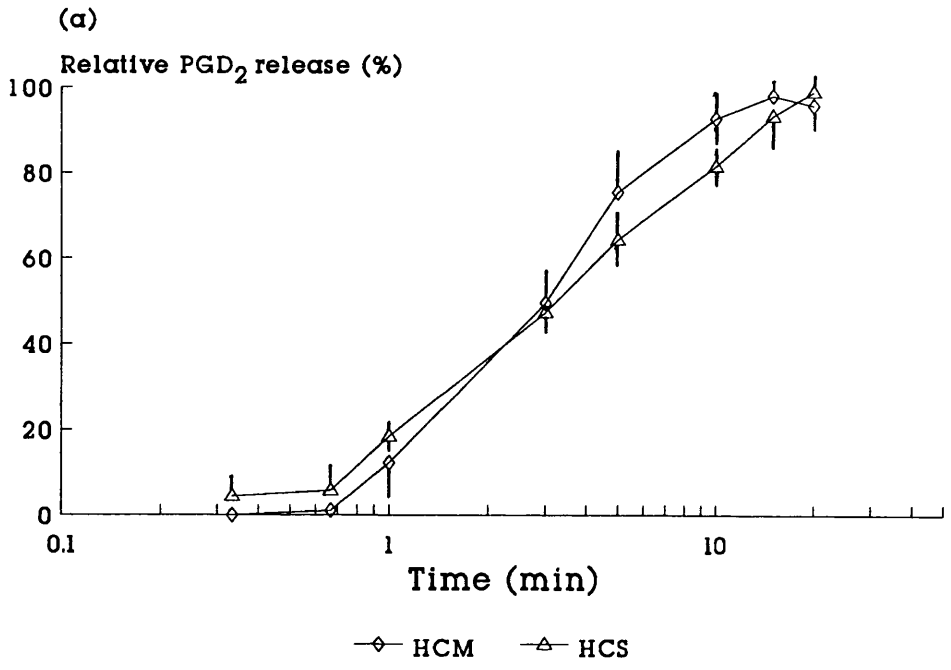


Fig 6.6 Kinetics of (a) PGD_2 release (n=3) and (b) LTC_4 release (n=3) induced by anti-human IgE from isolated human mast cells from the colonic mucosa (HCM) and colonic submucosa/muscle (HCS).



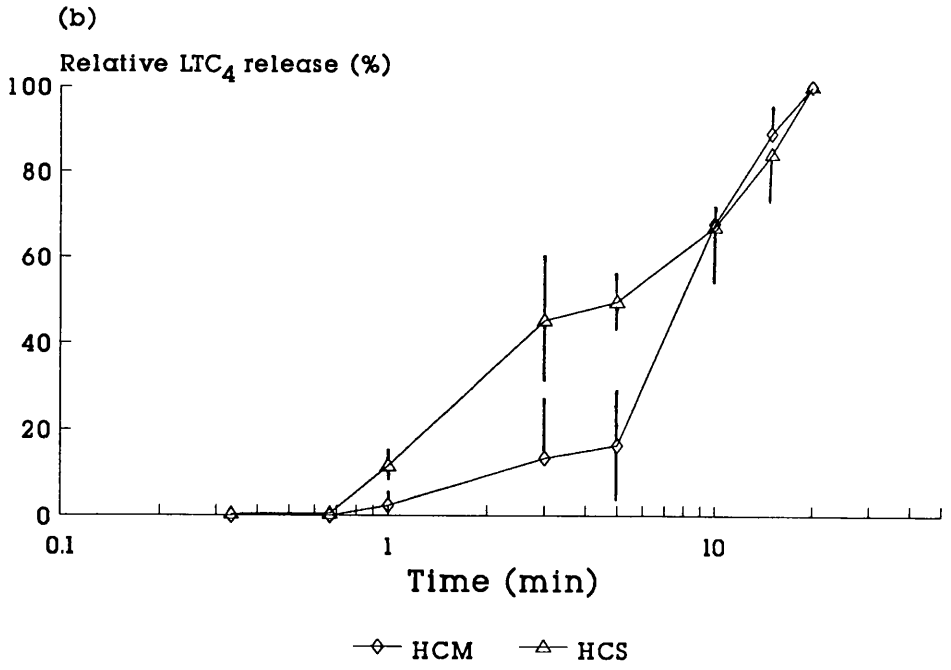


Fig 6.7 Correlation between histamine and PGD_2 release induced by anti-human IgE from isolated human mast cells from (a) colonic mucosa (n=3) and (b) colonic submucosa/muscle (n=3).

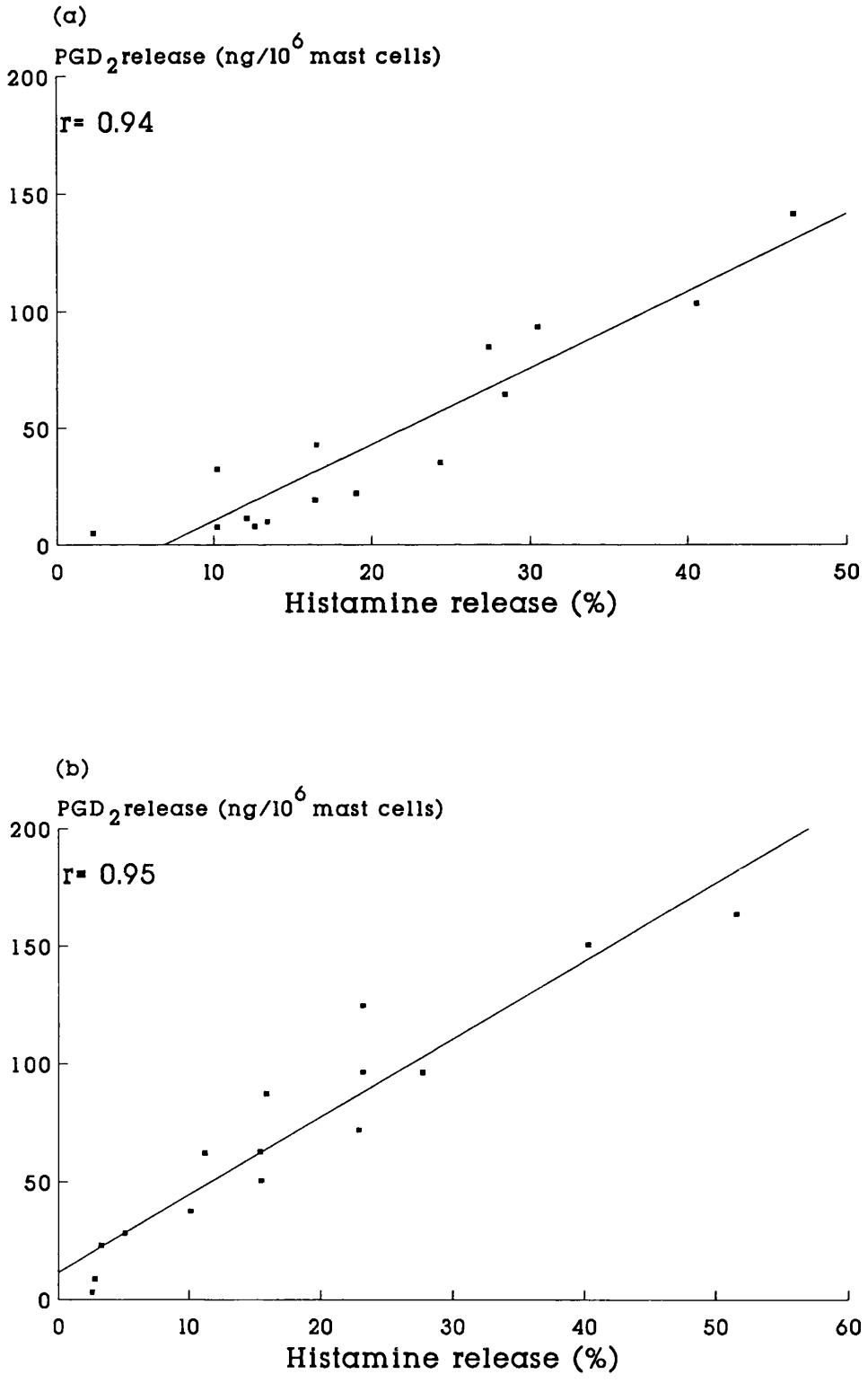


Fig 6.8 Correlation between histamine and LTC_4 release induced by anti-human IgE from isolated human mast cells from (a) colonic mucosa (n=3) and (b) colonic submucosa/muscle (n=3).

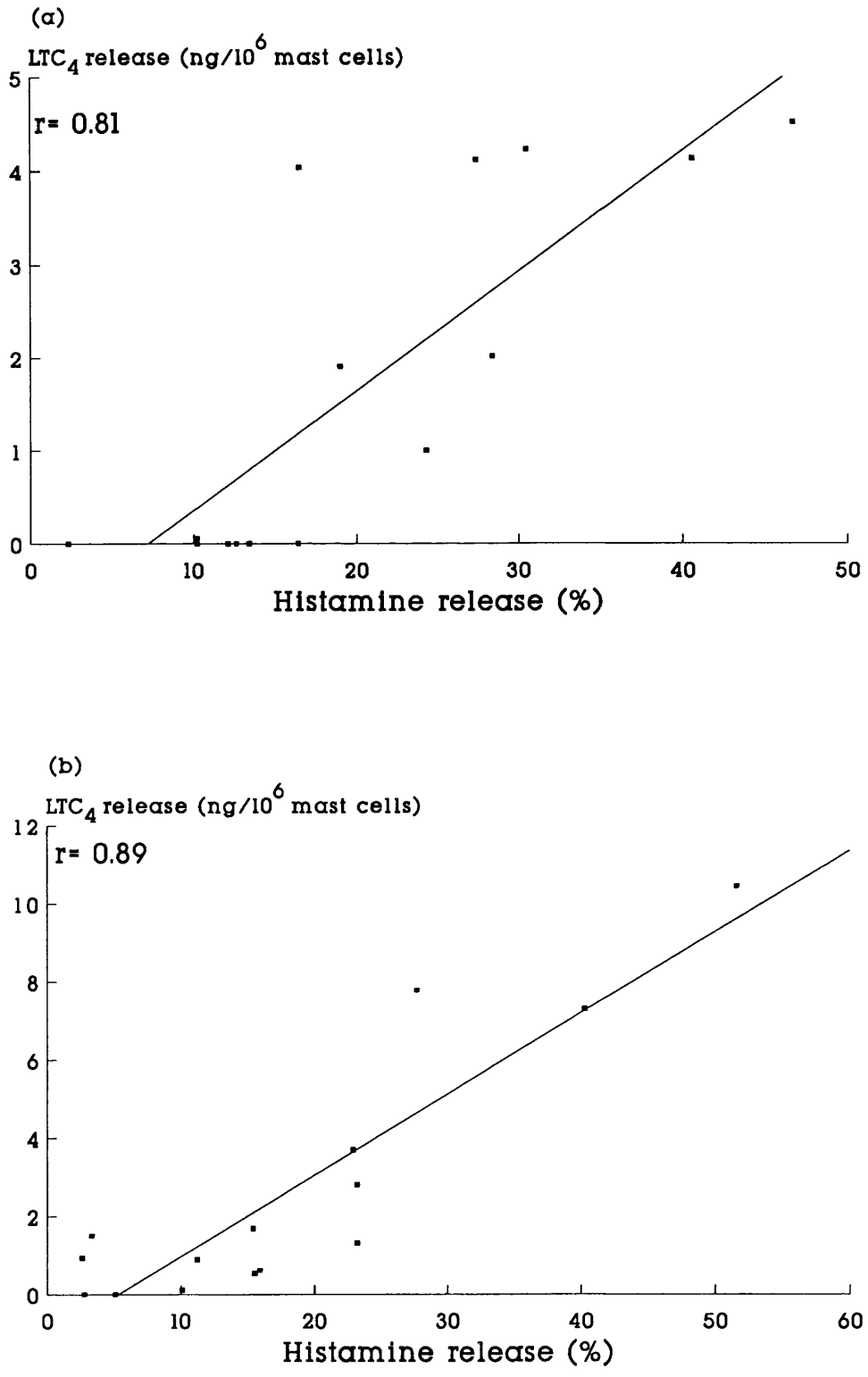
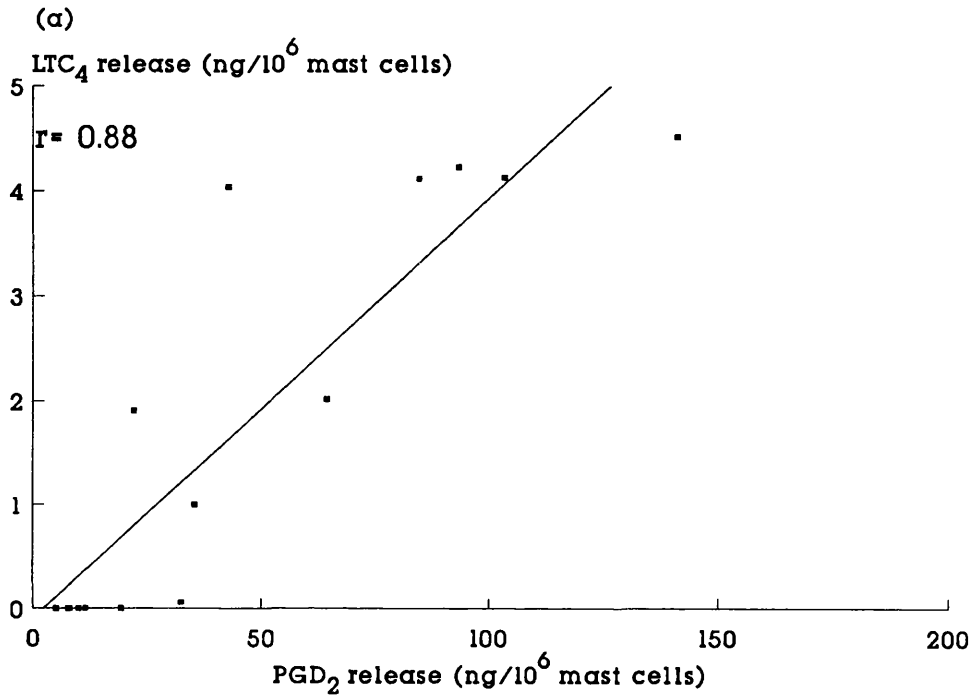


Fig 6.9 Correlation between PGD_2 and LTC_4 release induced by anti-human IgE from isolated human mast cells from (a) colonic mucosa (n=3) and (b) colonic submucosa/muscle (n=3).



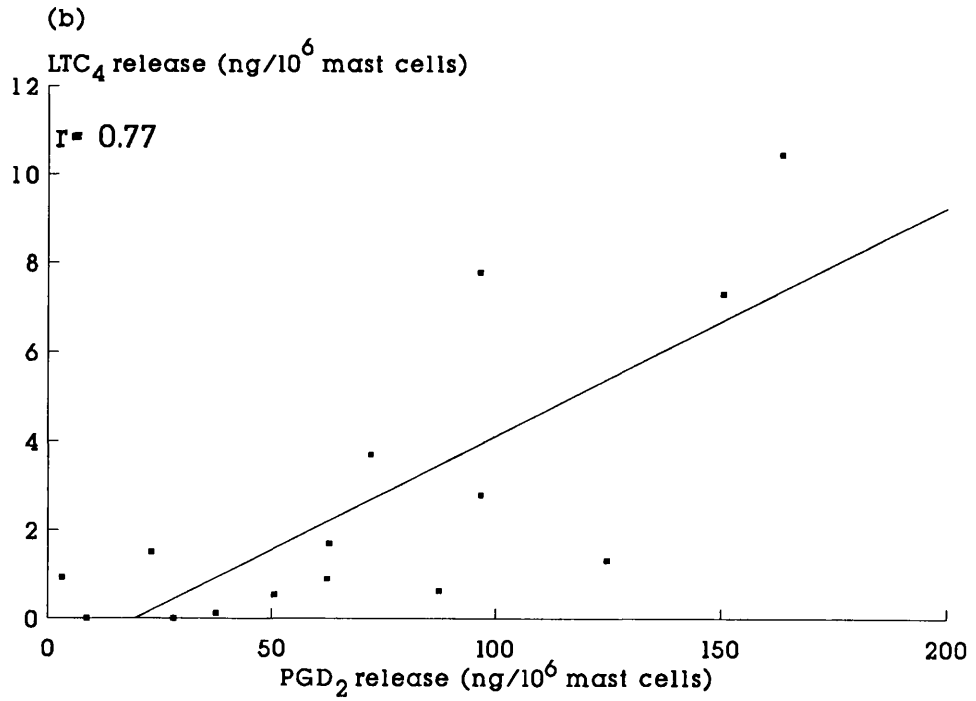
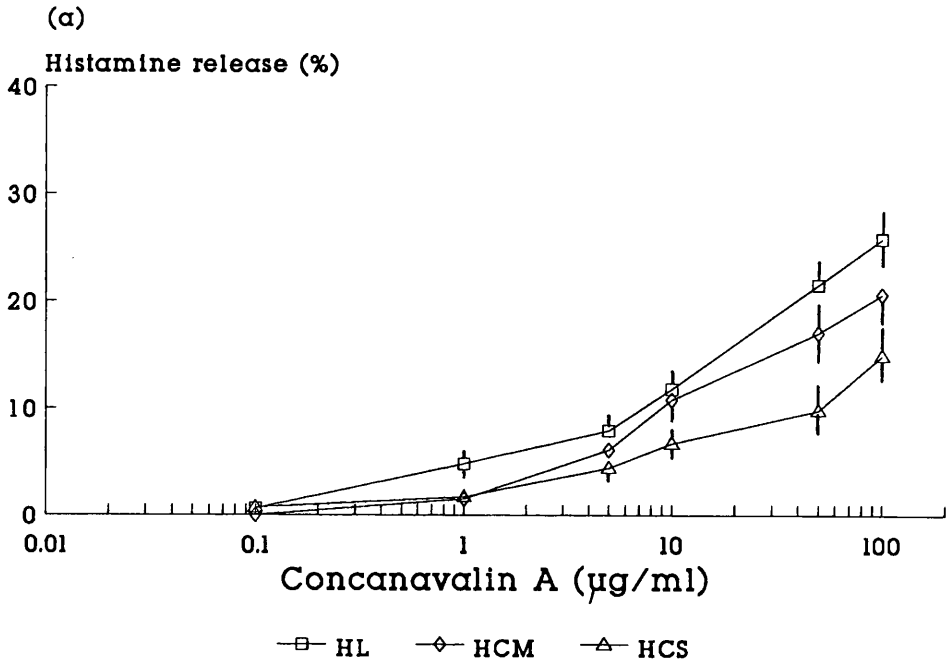


Fig 6.10 Histamine release induced by (a) concanavalin A (n=5-7) and (b) A23187 (n=4-8) from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS).



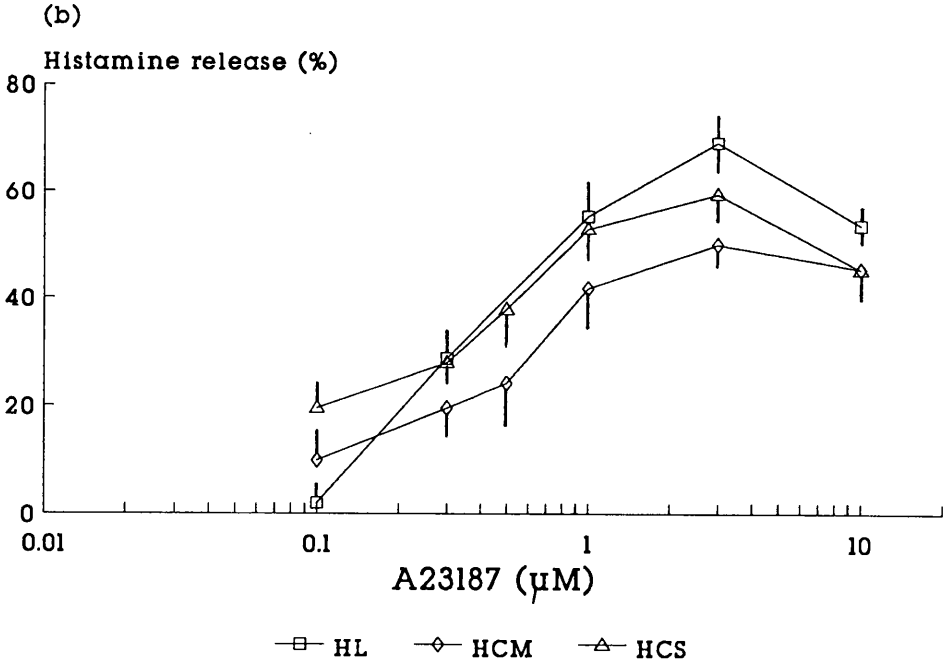
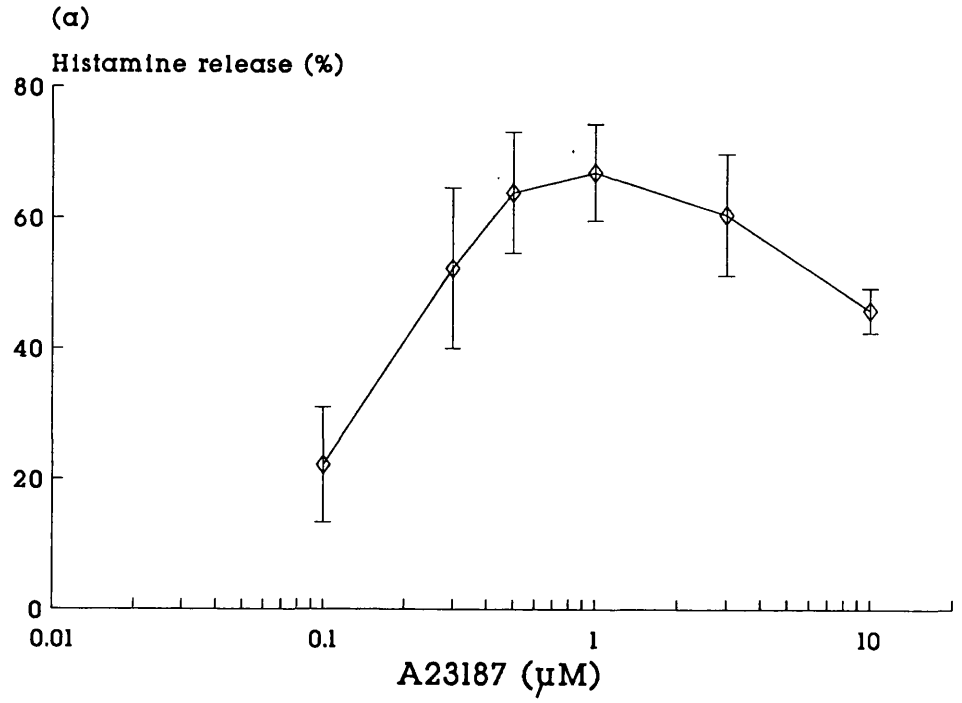


Fig 6.11 Histamine release induced by A23187 from isolated human mast cells from (a) colonic mucosa (n=3) and (b) colonic submucosa/muscle (n=3).



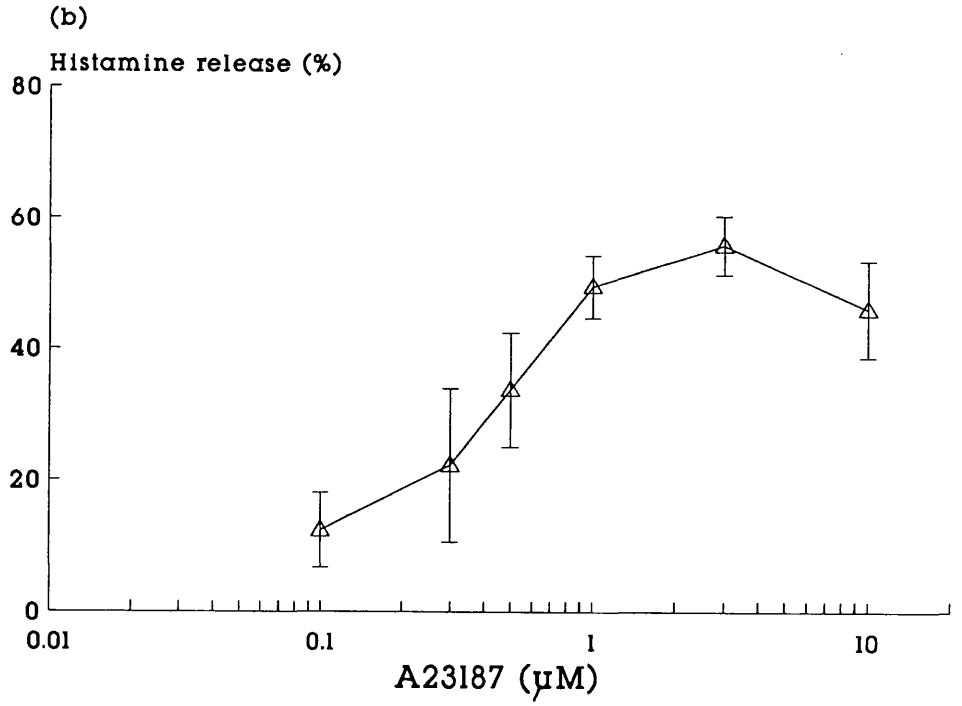
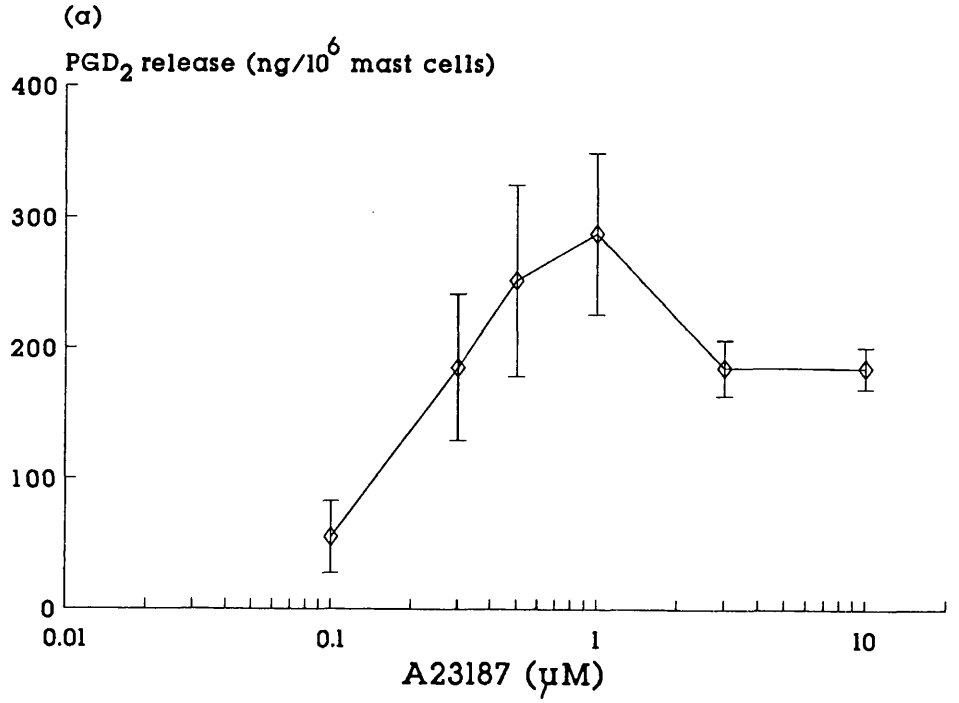


Fig 6.12 PGD_2 release induced by A23187 from isolated human mast cells from (a) colonic mucosa (n=3) and (b) colonic submucosa/muscle (n=3).



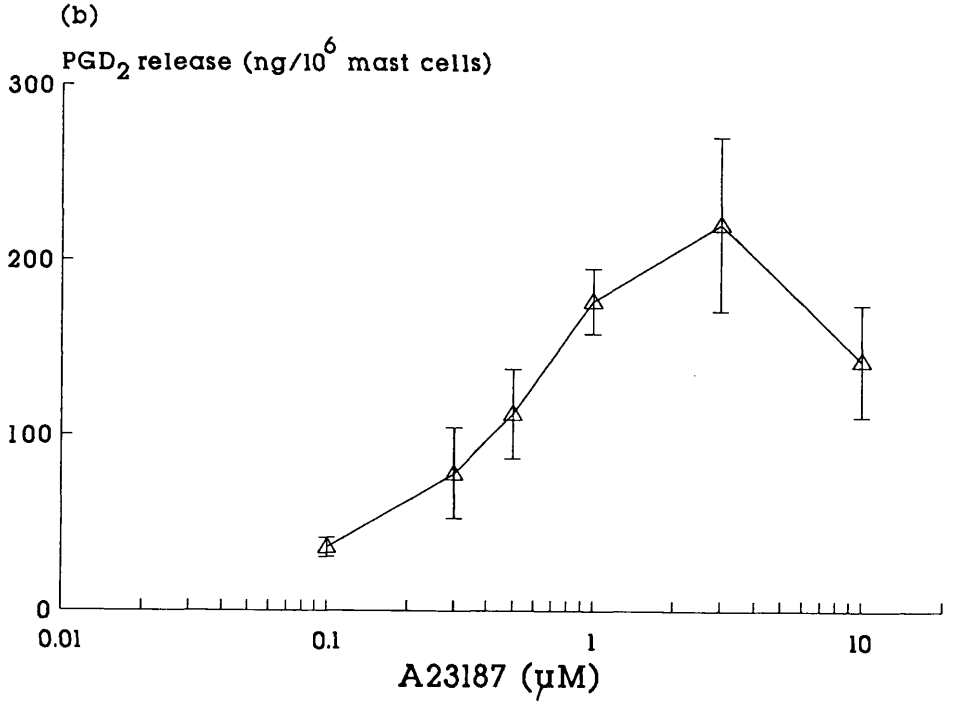
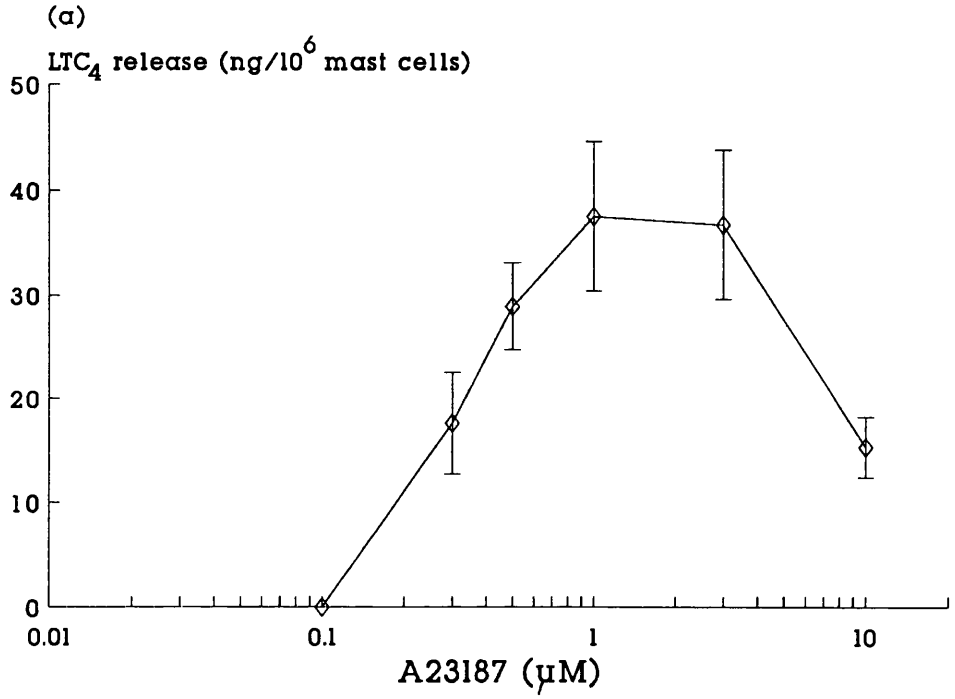


Fig 6.13 LTC_4 release induced by A23187 from isolated human mast cells from (a) colonic mucosa (n=3) and (b) colonic submucosa/muscle (n=3).



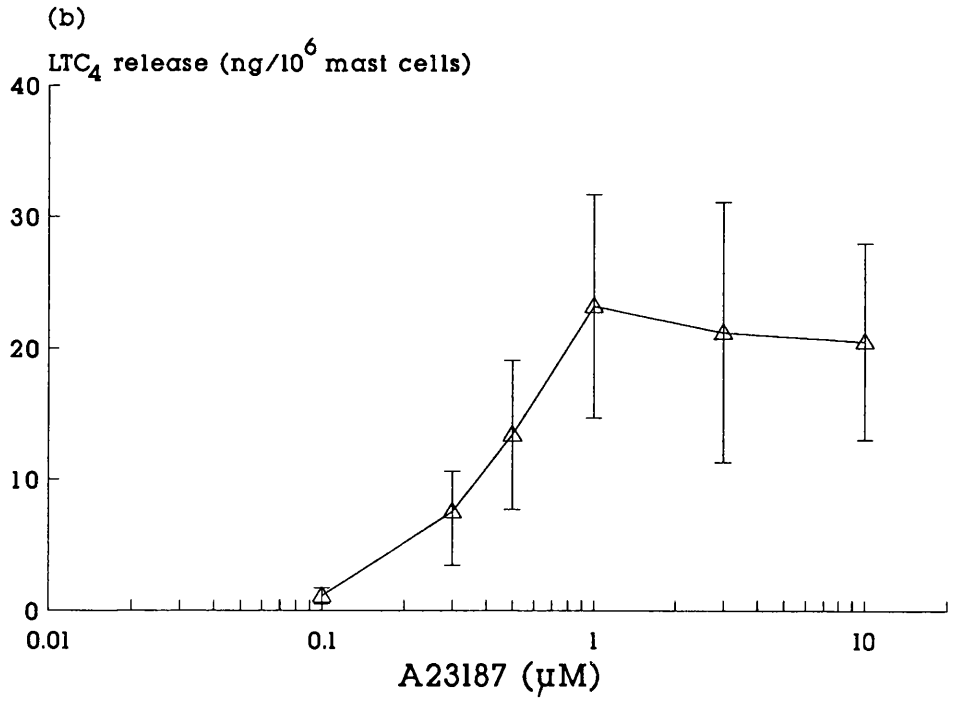
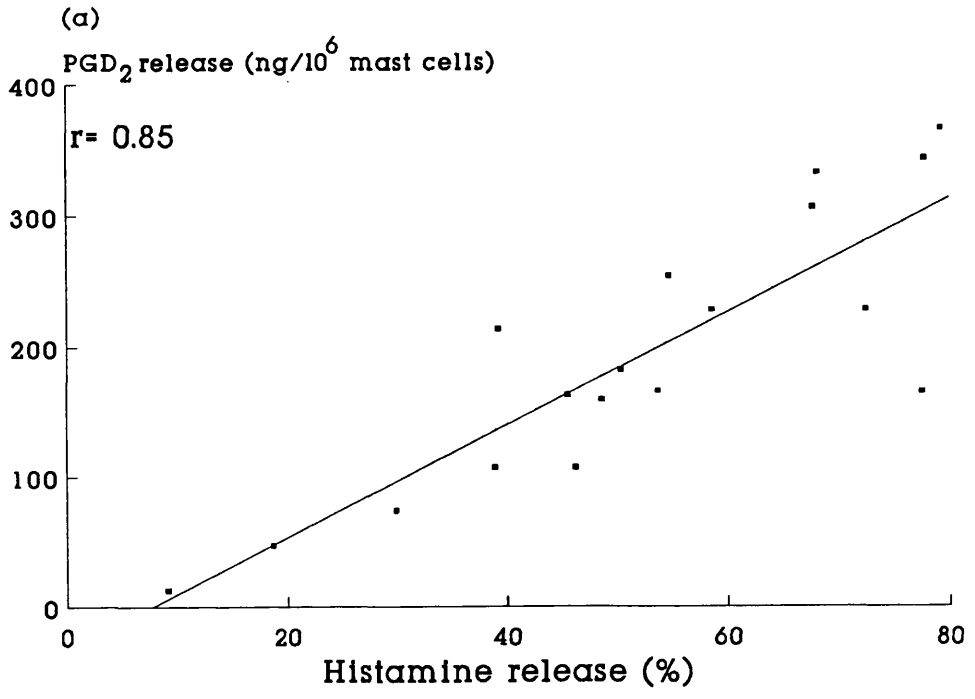


Fig 6.14 Correlation between histamine and PGD_2 release induced by A23187 from isolated human mast cells from (a) colonic mucosa (n=3) and (b) colonic submucosa/muscle (n=3).



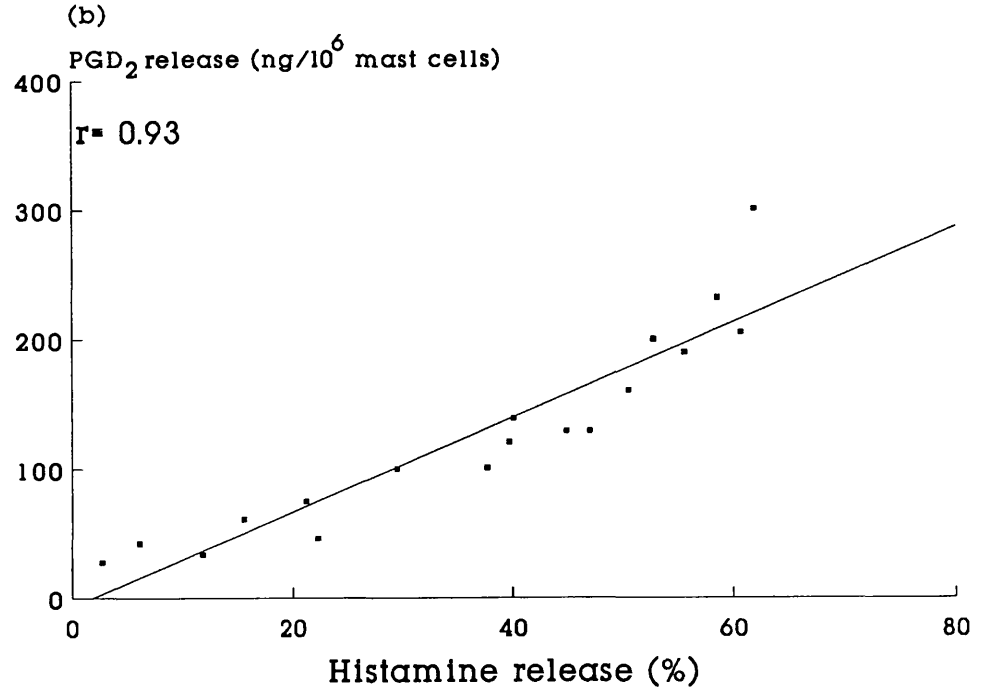
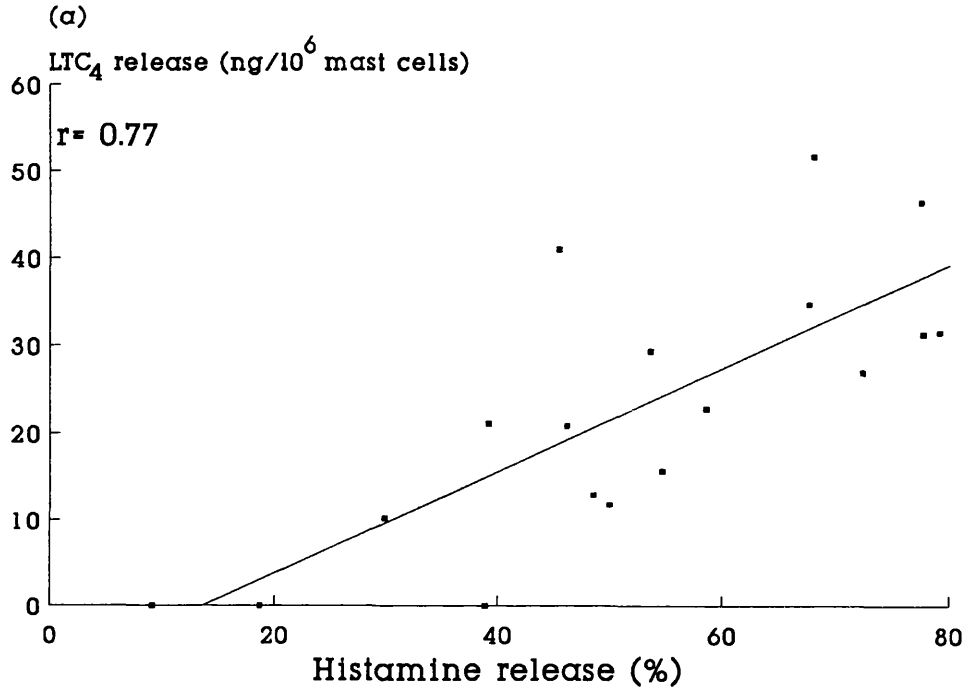


Fig 6.15 Correlation between histamine and LTC_4 release induced by A23187 from isolated human mast cells from (a) colonic mucosa (n=3) and (b) colonic submucosa/muscle (n=3).



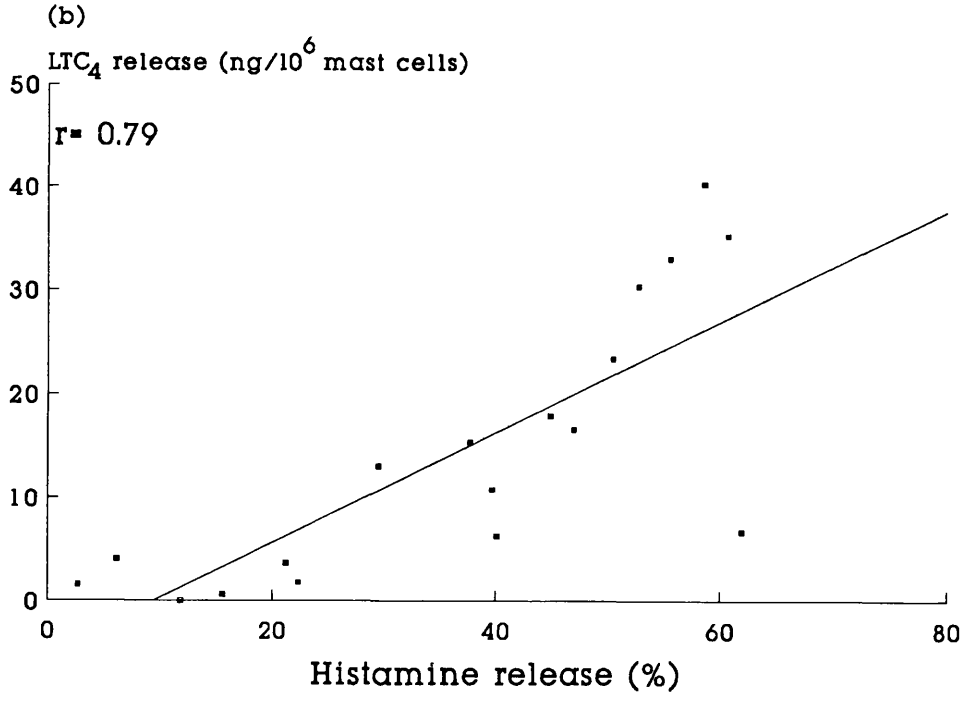
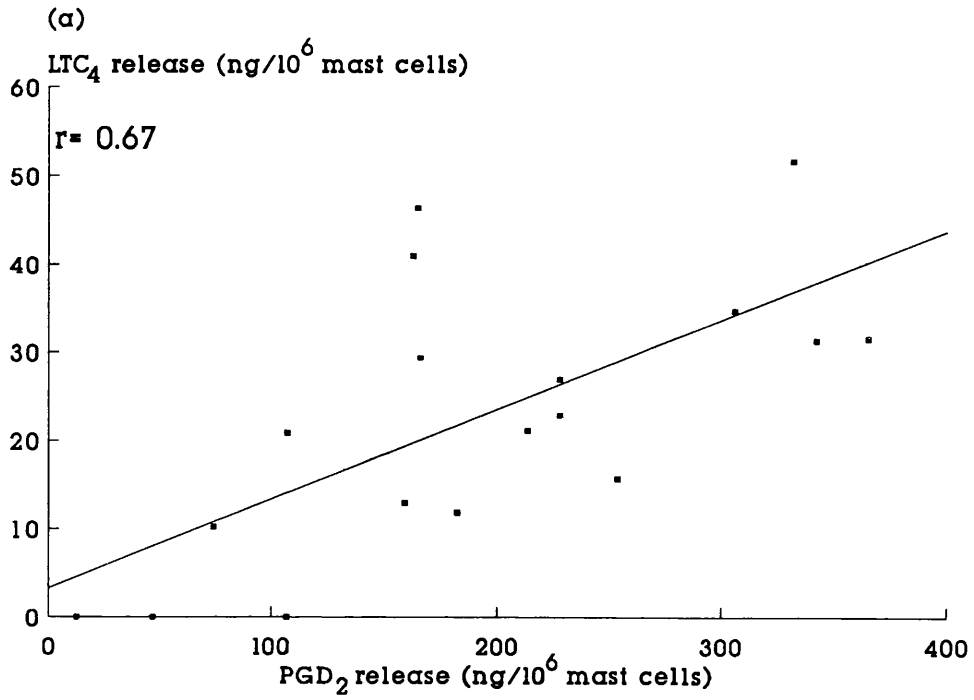


Fig 6.16 Correlation between PGD_2 and LTC_4 release induced by A23187 from isolated human mast cells from (a) colonic mucosa (n=3) and (b) colonic submucosa/muscle (n=3).



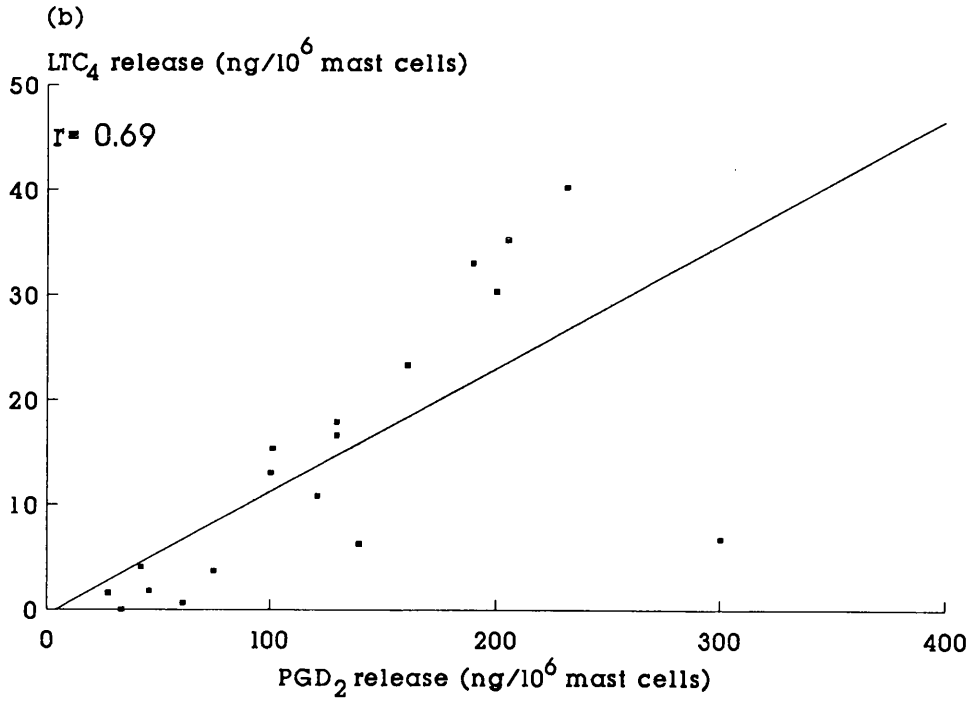
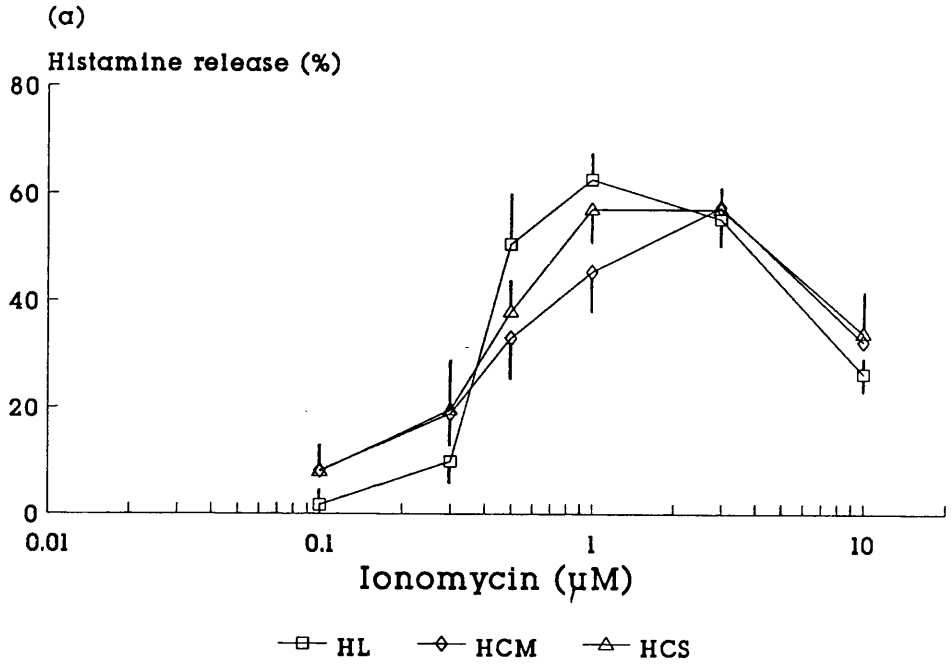


Fig 6.17 Histamine release induced by (a) ionomycin (n=4-6) and (b) bromo A23187 (n=4) from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS).



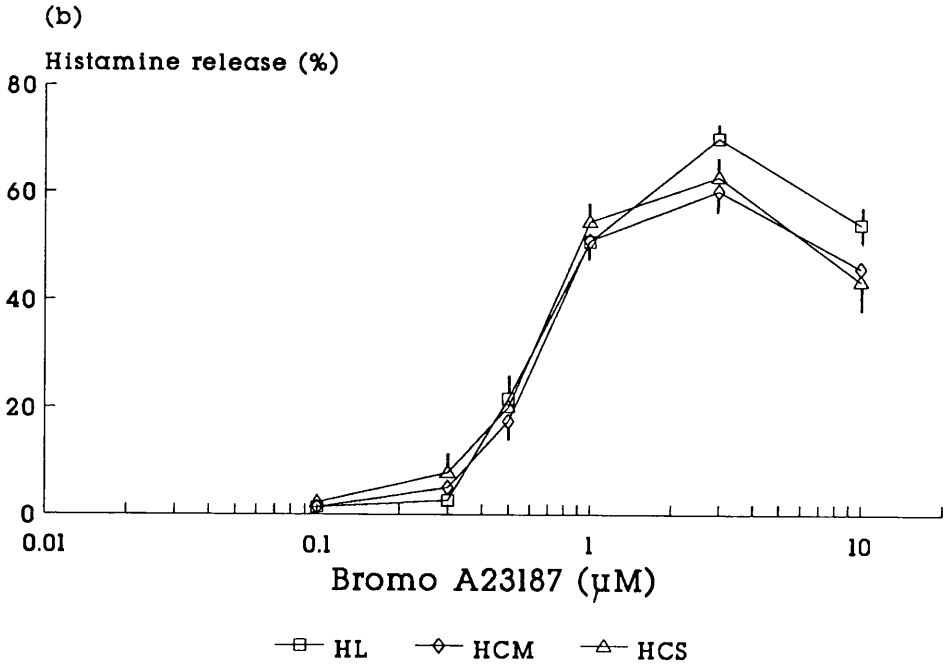
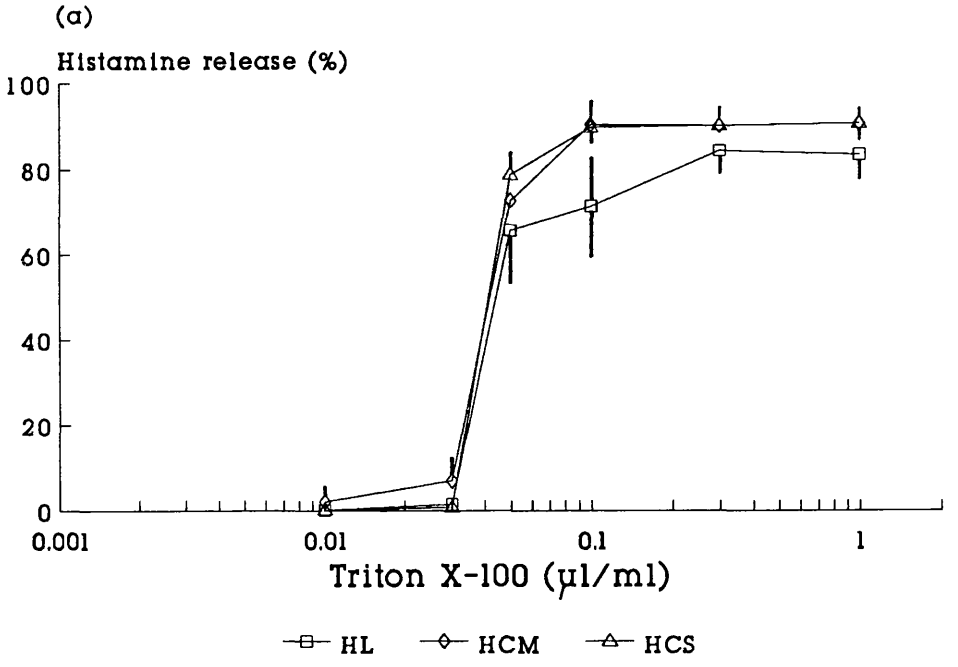


Fig 6.18 Histamine release induced by (a) Triton X-100 (n=4) and (b) Tween-20 (n=4) from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS).



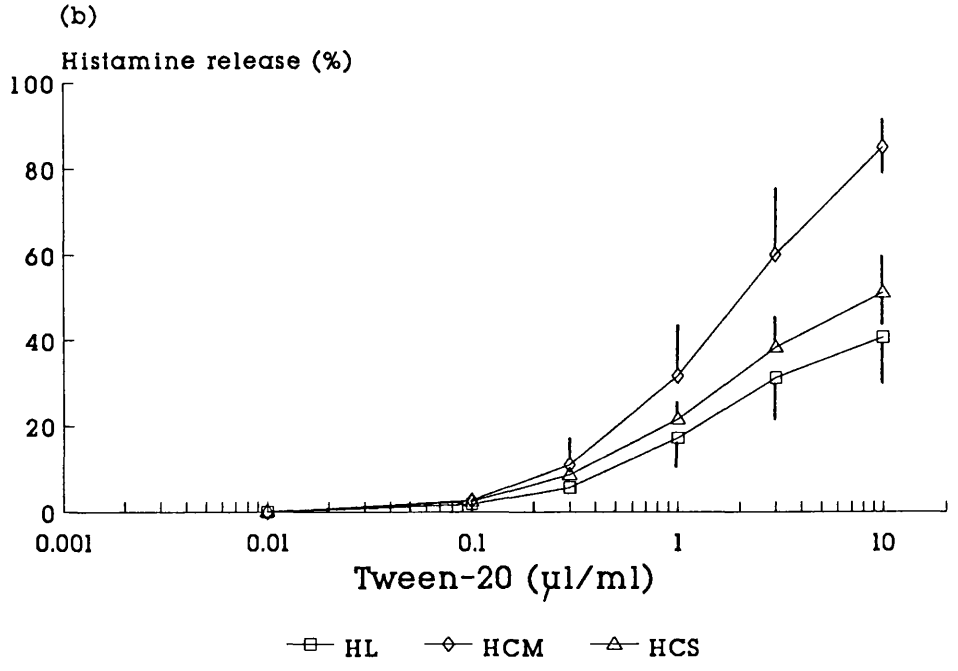
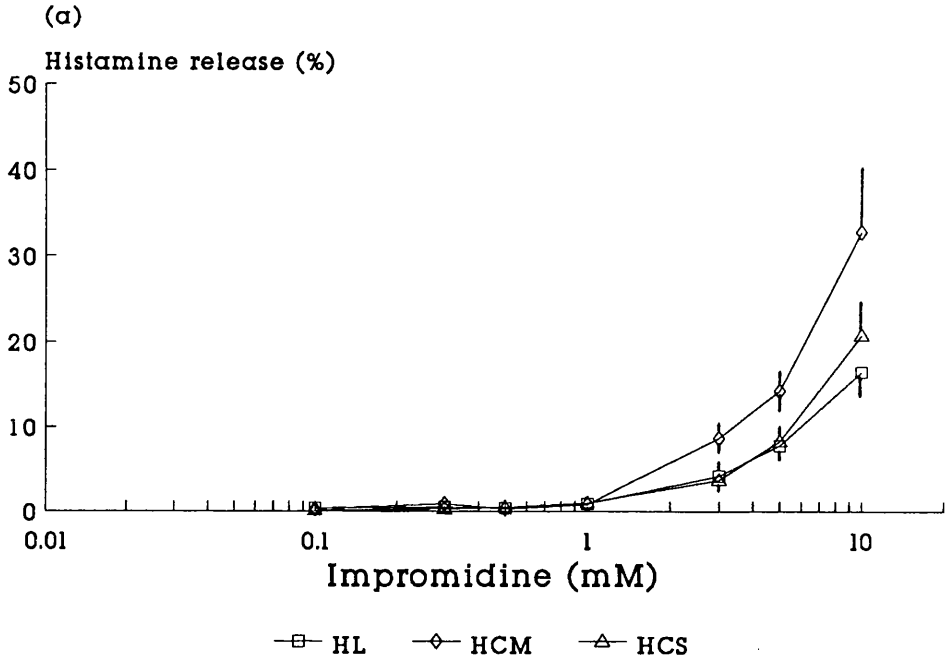


Fig 6.19 Histamine release induced by (a) impromidine (n=4) and (b) FMLP (n=4) from isolated human mast cells from the lung (HL), colonic mucosa (HCM), colonic submucosa/muscle (HCS), and human basophils (HB).



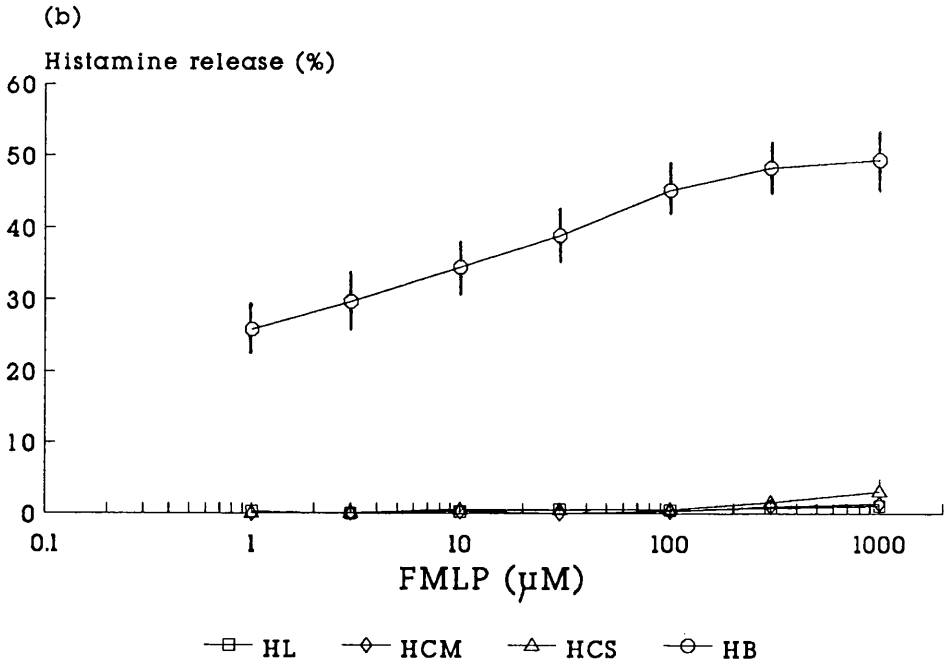


Table 6.1 Some basic properties of human mast cells isolated from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS).

	HL n=10	HCM n=10	HCS n=10
Dispersion efficiency (%)	15.2±1.1	18.0±1.2	5.5±0.7
Mast cells recovered x 10^6/g tissue	0.5±0.1	0.9±0.1	0.1±0.0
Mast cells (% total)	3.5±0.8	6.2±1.4	6.5±1.3
Viable cells (% total)	90.5±1.1	88.1±1.6	90.2±2.2
Spontaneous histamine release (%)	3.9±0.5	5.1±1.1	4.9±0.5
Histamine (pg/mast cell)	2.6±0.1	2.7±0.2	3.0±0.2
Formaldehyde sensitive (%)	88.5±1.8	91.3±1.3	69.6±3.8
Safranin positive (%)	0.0±0.0	0.0±0.0	0.0±0.0

Table 6.2 Histamine release from human mast cells induced by wheat germ lectin in the absence (a) or presence (b) of phosphatidylserine (15 μg/ml).

(a)

Concentration (μg/ml)	Histamine release (%) HL n=4	HCM n=4	HCS n=4
100	7.6±4.1	2.7±0.7	3.4±1.3
50	8.7±4.0	3.6±1.3	4.6±0.9
10	6.6±3.6	3.5±1.4	4.6±1.8
5	4.1±2.5	2.2±1.2	3.3±1.1
1	0.1±0.1	0.4±0.4	0.2±0.5

(b)

Concentration (μg/ml)	Histamine release (%) HL n=4	HCM n=4	HCS n=4
100	9.0±4.7	2.7±1.1	3.2±0.8
50	8.0±4.4	3.5±1.4	4.6±0.9
10	6.3±3.4	3.3±1.4	4.9±1.2
5	4.9±2.8	2.7±0.8	3.4±1.6
1	0.4±0.3	0.0±0.5	0.1±0.2

Table 6.3 Histamine release from human mast cells induced by anti-human IgG.

Concentration (dilution)	Histamine release (%) HL n=4	HCM n=4	HCS n=4
1/100	12.0±2.4	8.2±2.3	8.0±2.2
1/300	8.6±2.2	6.5±1.6	4.8±1.3
1/1000	5.1±1.5	4.8±1.2	3.0±0.9
1/3000	4.3±2.3	2.9±1.1	1.8±0.9
1/10000	2.7±1.9	1.4±0.6	0.4±0.2

Table 6.4 Histamine release from human mast cells induced by chlortetracycline.

Concentration (μM)	Histamine release (%) HL n=4	HCM n=4	HCS n=4
1000	5.6±1.1	7.4±1.1	6.4±0.5
300	2.8±0.6	2.9±0.5	2.9±0.3
100	1.2±0.4	0.8±0.8	1.0±0.3
30	0.3±0.2	0.0±0.7	0.3±0.4
10	0.5±0.3	-0.2±1.1	0.1±0.2
1	0.5±0.2	-0.3±1.1	-0.3±0.3

Table 6.5 Histamine release from human mast cells and human basophils (HB) induced by compound 48/80.

Concentration (μg/ml)	Histamine release (%) HL n=4	HCM n=5	HCS n=4	HB n=4
10	5.5±0.6	-0.6±0.8	2.0±1.1	0.2±0.2
1	2.4±0.3	-1.3±0.9	0.6±1.0	0.2±0.2
0.5	ND	-1.1±0.6	0.2±0.6	0.0±0.1
0.25	ND	-1.7±1.0	-0.4±0.4	0.0±0.1
0.1	1.8±0.2	-1.7±0.9	-0.5±0.7	0.0±0.0

Table 6.6 Histamine release from human colonic mast cells induced by compound 48/80.

Concentration (μg/ml)	Histamine release (%) HCM n=3	HCS n=3
10	2.3±1.1	4.3±3.3
1	1.3±0.8	1.4±1.0
0.5	0.8±0.4	0.5±0.4
0.25	0.6±0.5	0.5±0.3
0.1	-0.3±0.1	0.4±0.3

Table 6.7 PGD_2 release from human colonic mast cells induced by compound 48/80.

Concentration (μg/ml)	PGD_2 release (ng/10^6 mast cells) HCM n=3	HCS n=3
10	1.6±1.6	3.6±1.6
1	1.8±1.3	1.8±1.2
0.5	0.3±0.3	1.7±1.4
0.25	3.7±2.2	1.8±1.2
0.1	2.6±1.4	2.2±2.2

Table 6.8 LTC_4 release from human colonic mast cells induced by compound 48/80.

Concentration (μg/ml)	LTC_4 release (ng/10^6 mast cells) HCM n=3	HCS n=3
10	0.0±0.0	0.0±0.0
1	0.0±0.0	0.4±0.4
0.5	0.0±0.0	0.0±0.0
0.25	0.0±0.0	0.0±0.0
0.1	0.0±0.0	0.1±0.1

Table 6.9 Histamine release from human mast cells and human basophils induced by polymyxin.

Concentration (μg/ml)	Histamine release (%) HL n=4	HCM n=5	HCS n=4	HB n=4
100	1.6±0.3	2.4±1.2	1.9±0.3	6.3±1.3
33.3	0.5±0.3	0.3±0.4	1.3±0.6	ND
10	0.2±0.2	-0.6±0.4	0.5±0.3	0.6±0.1
1	0.3±0.3	-0.5±0.5	0.2±0.3	0.1±0.1
0.1	0.1±0.1	-0.6±0.5	-0.2±0.2	0.2±0.2

Table 6.10 Histamine release from human mast cells and human basophils induced by substance P.

Concentration (μM)	Histamine release (%) HL n=4	HCM n=5	HCS n=4	HB n=4
50	1.5±0.5	2.7±1.1	4.1±0.3	3.2±0.6
10	0.1±1.2	0.1±1.2	1.8±0.4	1.1±0.4
5	-0.6±0.8	-0.6±0.8	0.3±0.1	0.4±0.3
1	-0.9±0.9	-0.9±0.5	-0.3±0.3	0.5±0.2
0.1	-0.3±0.6	-0.2±0.6	-0.1±0.5	0.4±0.3

Table 6.11 Histamine release from human mast cells and human basophils induced by $SP_{1-4}C_{12}$.

Concentration (μM)	Histamine release (%) HL n=4	HCM n=5	HCS n=4	HB n=4
10	3.0±0.8	3.2±1.4	1.4±0.2	1.5±0.4
1	1.8±0.4	1.5±1.1	0.3±0.1	1.1±0.3
0.1	0.8±0.1	0.8±1.1	0.5±0.4	0.8±1.1
0.01	0.4±0.2	0.2±0.8	0.1±0.4	0.5±0.2
0.001	0.4±0.1	0.3±0.5	-0.4±0.1	0.3±0.1

Table 6.12 Histamine release from human mast cells and human basophils induced by polylysine.

Concentration (μg/ml)	Histamine release (%) HL n=4	HCM n=5	HCS n=4	HB n=4
100	4.3±0.5	2.0±0.7	4.5±2.5	12.5±1.0
33.3	2.2±0.5	2.0±0.9	3.0±0.9	ND
10	3.1±0.5	0.0±0.7	1.0±0.4	9.8±0.3
1	2.2±0.7	-0.8±0.5	0.1±0.4	0.1±0.2
0.1	1.1±0.4	-1.1±0.3	0.1±0.8	0.1±0.1

Table 6.13 Histamine release from human mast cells and human basophils induced by polyarginine.

Concentration (μg/ml)	Histamine release (%) HL n=4	HCM n=4	HCS n=4	HB n=4
100	5.2±0.3	7.7±1.4	10.2±0.8	48.6±1.6
33.3	3.6±0.5	8.7±2.8	8.6±1.5	ND
10	2.5±0.2	10.3±3.4	9.7±2.1	44.0±1.2
1	1.9±0.3	-0.2±0.5	0.8±0.5	0.3±0.2
0.1	1.1±0.3	-0.7±0.7	0.4±0.2	0.3±0.1

Table 6.14 Histamine release from human mast cells and human basophils induced by clinical dextran in the absence (a) or presence (b) of phosphatidylserine (15 μg/ml).

(a)

Concentration (mg/ml)	Histamine release (%) HL n=4	HCM n=4	HCS n=4	HB n=4
10	0.1±0.2	0.5±0.4	-0.2±0.5	0.3±0.2
3	0.2±0.4	0.9±0.8	0.3±0.8	0.4±0.5
1	0.1±0.4	-0.4±0.6	0.2±0.7	-0.2±0.2
0.3	0.8±0.2	0.0±0.5	-0.8±0.6	0.0±0.2
0.1	0.7±0.3	0.0±0.3	-0.1±0.5	-0.3±0.2

(b)

Concentration (mg/ml)	Histamine release (%) HL n=4	HCM n=4	HCS n=4	HB n=4
10	0.9±0.5	0.3±0.6	0.6±0.3	0.6±0.3
3	0.9±0.3	0.1±0.5	0.9±0.7	0.6±0.4
1	0.6±0.4	1.1±1.8	0.5±0.2	0.1±0.3
0.3	0.7±0.4	-0.8±0.7	0.4±0.4	-0.2±0.3
0.1	1.1±0.8	-0.8±0.6	0.9±0.7	0.0±0.2

CHAPTER SEVEN

EFFECTS OF ANTI-ALLERGIC COMPOUNDS ON HISTAMINE RELEASE FROM HUMAN COLONIC AND LUNG MAST CELLS

7.1 Introduction

Most allergic disorders have now been appreciated to be complex inflammatory processes involving the participation of a variety of different cell types. Of these, the mast cell has commanded a pre-eminent position both because of its ability to release a diversity of spasmogenic, chemotactic and inflammatory mediators, and in view of its strategic location at the portals of entry into the body of foreign substances. Thus, while the mast cell is widely distributed throughout the human body, it is found in the greatest numbers in the loose connective tissues of the bronchi, conjunctiva, gut, ear, nose, throat and skin. The mast cell is then uniquely placed to participate in allergic responses and, as such, has been incriminated in the aetiology and pathogenesis of asthma, rhinitis, conjunctivitis and inflammatory disorders of the gut and skin.

Studies on rat peritoneal mast cells have provided valuable information concerning the control and inhibition of mediator secretion. However, substantial evidence has now been accumulated to indicate that the effects of a given anti-allergic drug on rat mast cells are not necessarily representative of their potential effects in man [257,279,283,302,350]. Thus, in spite of the high activity of DSCG on rat serosal mast cells, the tachyphylaxis observed with the drug is totally in contrast with its prophylactic use in man. Moreover, these rat cells appear to be relatively unresponsive towards β-adrenoceptor agonists which are potent inhibitors in human lung. Given this, it is the aim of the present study to investigate the effects of a number of anti-allergic compounds on isolated human colonic and lung mast cells. Moreover, it will be interesting to see whether the functional differences observed in chapter five, for rat mast cells from different anatomical locations, also apply to these human cells.

7.2 Methods

All methods used in this study were as described in chapter two.

7.3 Results

Unless otherwise stated, histamine release in all cases was induced by anti-human IgE.

7.3.1 Effects of DSCG and related compounds

Both DSCG (fig 7.1) and nedocromil sodium (fig 7.5) produced a dose-dependent inhibition of anti-IgE-induced histamine release from human mast cells isolated from the lung parenchyma, colonic mucosa and colonic submucosa/muscle. The former compound was most potent against the colonic mucosal cell while the latter agent was most active against the lung parenchymal cell. Moreover, the two drugs exhibited tachyphylaxis against the lung parenchymal cell (figs 7.2 and 7.6), and to a lesser extent against the colonic submucosal/muscle cell (figs 7.4 and 7.8), while the inhibition against the colonic mucosal cell (figs 7.3 and 7.7) was maintained with preincubation.

Quercetin, a naturally occurring flavonoid structurally related to DSCG, was a potent inhibitor of anti-IgE-induced histamine release from all three mast cell preparations (fig 7.9). Another flavonoid, chrysin, also exhibited comparable inhibitory activity against the different cell types but the effects observed were slightly less pronounced as compared to quercetin (fig 7.10).

7.3.2 Effects of LU 48953 and MY 1250

LU 48953 (Knoll) suppressed the anti-IgE-induced histamine release from human lung parenchymal mast cells in a concentration-related manner (fig 7.11), and was equally active against mast cells from the two colonic portions. In total contrast to the chromones, LU 48953 exhibited no tachyphylaxis against any of the three mast cell preparations studied (figs 7.12-7.14).

MY 1250 (Bayer), a potent inhibitor of allergen-induced histamine release from rat peritoneal mast cells [chapter 5], had essentially no effect against the colonic submucosal/muscle cell (table 7.1). Weak inhibition, however, was observed with both lung parenchymal and colonic mucosal cells, after an initial 10 minutes preincubation, at the highest drug concentration used (10^{-4} M).

7.3.3 Effects of cAMP-active compounds

The cyclic nucleotide analogue Bu_2cAMP (fig 7.15) and the phosphodiesterase inhibitors theophylline (fig 7.16) and IBMX (fig 7.17) were potent inhibitors, in a dose-dependent fashion, of anti-IgE-induced histamine release from the three human mast cell preparations studied. These mast cells also responded to isoprenaline (fig 7.18) and salbutamol (fig 7.19), although the colonic mucosal cells appeared to be somewhat less responsive to the inhibitory effects of the β-adrenoceptor agonists.

7.3.4 Effects of histamine receptor directed compounds

The H_2-receptor antagonists cimetidine (fig 7.20) and ranitidine (fig 7.21), used clinically in the treatment of peptic ulcer disease, were powerful inhibitors of anti-IgE-induced histamine release from human colonic mucosal mast cells. The former compound also exhibited comparable activity against these cells from the lung parenchyma and colonic submucosa/muscle. In contrast, the latter agent was less effective on these two mast cell preparations, particularly against those from the lung parenchyma.

Dimaprit (fig 7.22), an H_2-receptor agonist, also showed considerable inhibitory activity against the colonic mucosal cells, but like ranitidine, was less active against cells from the lung parenchyma and colonic submucosa/muscle.

7.4 Discussion

The results of the previous chapter have clearly demonstrated that, apart from some differences in their histochemical properties, isolated human mast cells from the

lung parenchyma, colonic mucosa and colonic submucosa/muscle were extremely similar, notably in their responses to a number of immunological and non-immunological secretagogues and in the types of eicosanoids generated upon activation. The present study has extended this investigation by examining the effects of a number of anti-allergic compounds.

DSCG was first introduced as an anti-allergic drug in the United Kingdom in 1967 [319,379], and has been employed in the treatment of a number of allergic disorders ranging from asthma to food allergy. The clinical utility of the drug is undoubtedly complex but has been generally attributed, at least in part, to its ability to inhibit mediator release from tissue mast cells. However, as has been clearly demonstrated in chapters three and five and also from other published reports [257,279,283,302,350,380], the chromone provides a particularly striking example of the selectivity of a given mast cell stabilizing compound. As is well known, the drug is a very potent inhibitor of immunologically induced histamine release from rat peritoneal and pleural mast cells. In contrast, the compound is significantly less active against peritoneal cells from the hamster and totally ineffective against those cells from the mouse. Moreover, bone marrow-derived mouse mast cells, tissue mast cells from the guinea pig and rat, intestinal mast cells of nonhuman primates and human basophils are only weakly responsive or essentially insensitive towards the chromone.

The effect of DSCG on isolated human mast cells has been the subject of intense research. Indeed, it is now generally accepted that higher concentrations of the chromone are required to inhibit IgE-dependent histamine release from these cells as compared to rat serosal mast cells. Leung et al [283-285,323] and Church and Hiroi [215] have shown that DSCG is active on dispersed human lung parenchymal mast cells at concentrations greater than 10^{-6} M, producing a maximum inhibition of histamine release of around 30 %. This effect is, however, significantly reduced when the cells are first preincubated with the drug before challenge. In addition to its effect on parenchymal cells, DSCG also inhibits histamine secretion from lung mast cells recovered by bronchoalveolar lavage (BAL) [283-285,323]. Moreover, these cells are significantly more sensitive to the chromone than the parenchymal cell and, more importantly, exhibit no tachyphylaxis upon preincubation with the drug. As mentioned

in chapter three, tachyphylaxis is not observed with this drug in the treatment of asthma, suggesting that the site of action in vivo is the lavage mast cell, which is presumably the first to encounter allergen, rather than the mast cells in the lung parenchyma.

The effect of DSCG on isolated human intestinal mast cells, unlike their lung counterparts, is more confusing. Fox et al [381] reported the chromone to have no effect on human intestinal mucosal mast cells whereas Befus et al [78] observed weak inhibition of histamine release from these cells at high drug concentrations. In the former study, DSCG was preincubated with the cells for 5 minutes before challenge whereas in the latter study, the drug was added concurrently with the stimulus and the effect of preincubation was not assessed. The results of the present study were in total contrast to these two previous reports. Isolated human mast cells from the colonic mucosa were more sensitive to the chromone than the lung parenchymal cell. Moreover, the inhibition against the colonic mucosal cell was maintained or sometimes even increased with preincubation, suggesting the cell in this respect to be functionally similar to the BAL cell. Indeed, the ability of DSCG to inhibit IgE-dependent histamine release from intestinal mast cells may constitute a component of its therapeutic effect in allergic and inflammatory diseases of the human intestine; this theme will be discussed further in chapter eight. In keeping with previous reports, DSCG produced a maximum inhibition of histamine release of around 30 % against the lung parenchymal mast cell, with the effect being significantly reduced after a 10 minutes preincubation with the drug before challenge. Mast cells from the colonic submucosa/muscle were similarly affected, but the tachyphylactic effect observed was strikingly less than the parenchymal cell.

Nedocromil sodium is a pyranoquinoline dicarboxylic acid with structural similarities to DSCG and is thought to exert its effect via a similar mechanism [322]. In human lung parenchymal and BAL mast cells, nedocromil sodium is more potent than DSCG in inhibiting IgE-dependent histamine release [283-285,323]. In common with DSCG, rapid tachyphylaxis to nedocromil sodium occurs in parenchymal cells whereas preincubation does not alter its efficacy in BAL mast cells. In contrast to the lung, this drug was reported to be ineffective in inhibiting histamine release from

dispersed intestinal mucosal mast cells when added concurrently with the challenge [78]. The results of the present study have confirmed the reported effects of nedocromil sodium on lung parenchymal mast cells. However, in contrast to the results of Befus et al [78], nedocromil sodium was also active against the colonic mucosal mast cell, although the maximum inhibition achieved was lower than with the lung parenchymal cell. Finally, mast cells from the colonic submucosa/muscle were similarly affected as compared to their mucosal counterparts with the exception that tachyphylaxis was observed, but the effect was much less pronounced than the lung parenchymal cell.

It is important to mention that the apparent activity of the chromones varies markedly and inversely with the magnitude of the induced secretory response [283]. This phenomenon has important clinical implications since, for purely practical reasons of measurement, studies in vitro necessarily use induced immunological releases of histamine in the range of 15-40 %. Secretion of this magnitude probably never occurs in vivo, and the amount of histamine released in even the most severe asthmatic attack is likely to be less than 1 % of the total lung content of the amine. Under these conditions, the potency of both nedocromil sodium and DSCG is likely to be considerably greater than that measured by in vitro experiments.

The naturally occurring plant flavonoid, quercetin, is structurally related to DSCG and, along with a number of other flavonoids, has been demonstrated to inhibit histamine release from mast cells and basophils [331,362,363]. Indeed, in chapter five, this compound was shown to suppress potently allergen-induced histamine release from rat mast cells derived from different anatomical locations. In the present study, and in agreement with the results of Befus et al [78], quercetin essentially completely inhibited anti-IgE-induced histamine release from human colonic mucosal mast cells. Mast cells derived from the lung parenchyma and colonic submucosa/muscle were also similarly affected. The other flavonoid used in this study, chrysin, also showed comparable inhibitory activity against all three mast cell preparations but the effect observed was less pronounced than in the case of quercetin. Finally, the precise mechanism whereby the flavonoids prevent histamine release, like the chromones, is still uncertain, but it has been shown that these compounds are capable of inhibiting a number of key enzymes involved in the secretory process [362-366].

LU 48953 (Knoll) and MY 1250 (Bayer) were each synthesized with the aim of producing a compound with anti-allergic activity and which may be of clinical use. In the present study, in experiments carried out in conjunction with the chromones, LU 48953 dose-dependently inhibited anti-IgE-induced histamine release from human colonic mucosal mast cells, with the maximum inhibition achieved being similar to that observed for DSCG. Moreover, the compound was also equally active against mast cells derived from the lung parenchyma, BAL (H.Y.A. Lau, personal communication) and colonic submucosa/muscle. Most importantly, LU 48953 exhibited no tachyphylaxis against any one of these mast cell preparations. Therefore, in the light of the above results, tests should be carried out on other in vitro and in vivo systems to establish its full spectrum of activity. In contrast to LU 48953, MY 1250 was essentially inactive as an inhibitor of histamine release when added concurrently with challenge. Weak inhibition, however, was observed against both lung parenchymal and colonic mucosal mast cells, after an initial 10 minutes preincubation, at the highest drug concentration used (10^{-4} M).

Agents which elevate intracellular levels of cAMP have traditionally been associated with the inhibition of mediator release from the mast cell. This concept has, however, been questioned by the findings of chapter five and also by other published reports in the literature [219-221,257] which showed that β-adrenoceptor agonists are ineffective in inhibiting histamine release from rat mast cells derived from various anatomical locations. The situation with regard to human mast cells is more in line with the original concept. In vitro, the β-adrenoceptor agonists isoprenaline and salbutamol inhibit histamine release from human lung fragments [382] as well as isolated human lung parenchymal mast cells [118,215,383,384]. In addition, both compounds are also capable of suppressing the generation of the eicosanoids PGD_2 and LTC_4 from the latter system [215,383,384]. Moreover, they also demonstrate comparable activity against isolated human mast cells from the intestinal mucosa [78], skin [385] and BAL [118,339]. The results of the present study are in general agreement with these findings. Both isoprenaline and salbutamol inhibited, in a dose-dependent fashion, anti-IgE-induced histamine release from isolated mast cells from the lung parenchyma, colonic mucosa and colonic submucosa/muscle. It should be noted that the maximum inhibition produced by the two compounds rarely exceeds 60 % and this may imply that a

fraction of the immunologically released histamine is insulated from β-adrenergic modulation.

Apart from activating adenylate cyclase, intracellular levels of cAMP can also be raised by a number of other methods, notably by application of appropriate analogues of the nucleotide, or by preventing the breakdown of cAMP by inhibition of phosphodiesterase with methylxanthines. Indeed, these compounds have been found to be potent inhibitors of histamine release from isolated mast cells from a number of different species including man [76,78,221-223,257,302]. In keeping with this theme, the present study has demonstrated that three such agents, namely Bu_2cAMP, theophylline and IBMX all exhibited considerable inhibitory activity against the three human mast cell preparations studied.

The H_2-receptor agonist dimaprit and the corresponding antagonists ranitidine and cimetidine were found to block, in varying degrees, anti-IgE-induced histamine release from isolated human mast cells from the lung parenchyma, colonic mucosa and colonic submucosa/muscle. The latter compound was essentially equiactive on all three mast cell preparations whereas the former two agents were most potent against the colonic mucosal cell. The precise mechanism of these effects is not clear, but it has been proposed [342,367] that the inhibitory action of histamine receptor directed ligands is due to the intercalation of these compounds into the lipid bilayer in such a way as to stabilize the mast cell membrane, thereby preventing the functional changes involved in exocytosis. Indeed, the differential sensitivities of human mast cells towards these compounds may indicate a difference in the structure of the cell membrane.

The findings of chapter six have indicated that human mast cells recovered from the enzymic dispersion of the lung parenchyma, colonic mucosa and colonic submucosa/muscle are functionally similar in their responses to a variety of immunological and non-immunological secretagogues. The present study has demonstrated subtle variations between these human mast cells in their reactivities towards a number of anti-allergic compounds. However, although clearly evident, these differences are much less sharply defined than in the rodent.

Fig 7.1 Effect of disodium cromoglycate (DSCG) on anti-IgE-induced histamine release from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS). The drug was added simultaneously with stimulus to cells. The control releases were 29.9±4.5 %, n=4, HL; 14.7±3.1 %, n=6, HCM and 22.4±5.8 %, n=7, HCS.

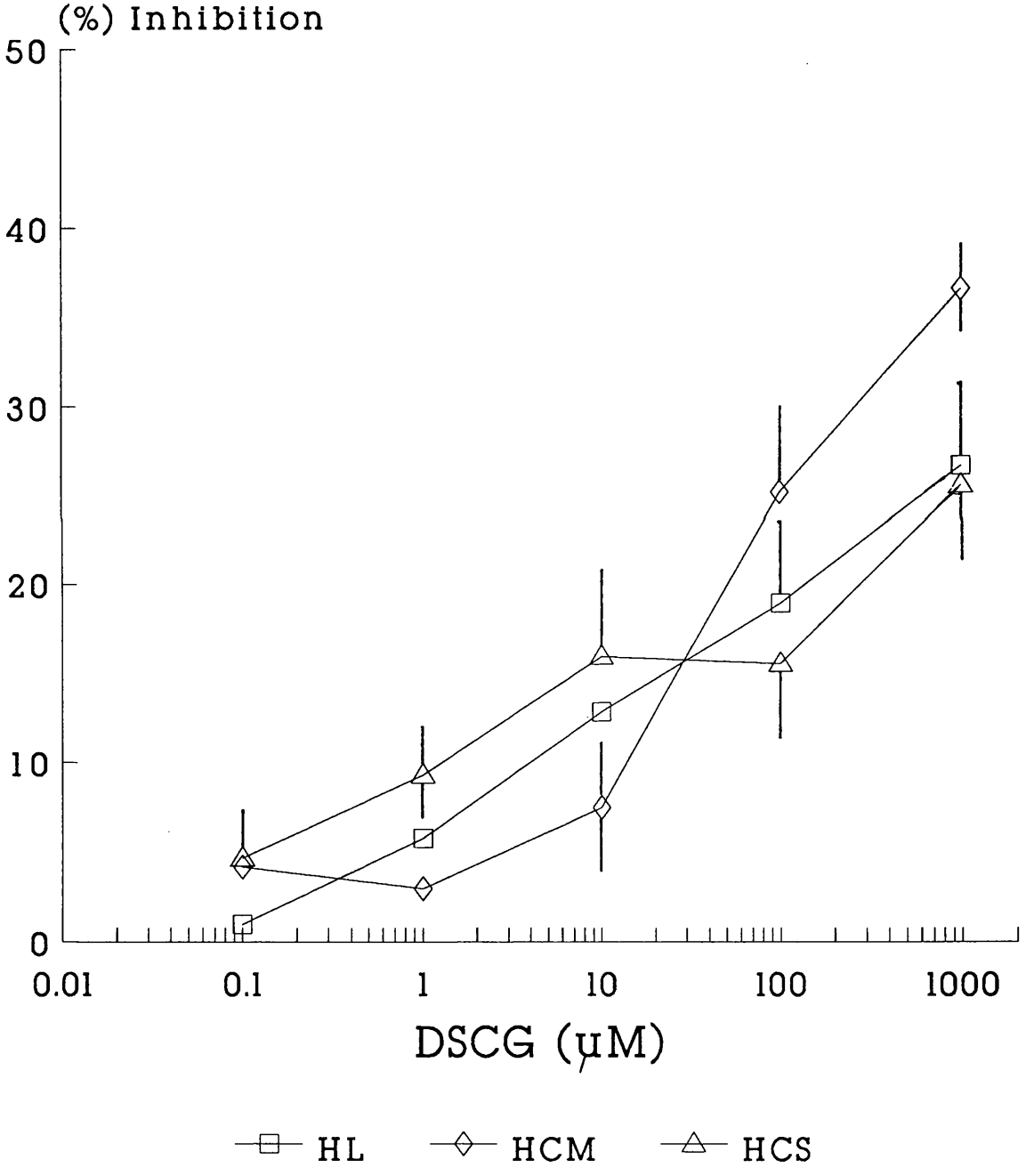


Fig 7.2 Effect of disodium cromoglycate (DSCG) on anti-IgE-induced histamine release from isolated human lung mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 29.9±4.5 % (0') and 29.7±4.5 % (10') respectively, n=4, paired data.

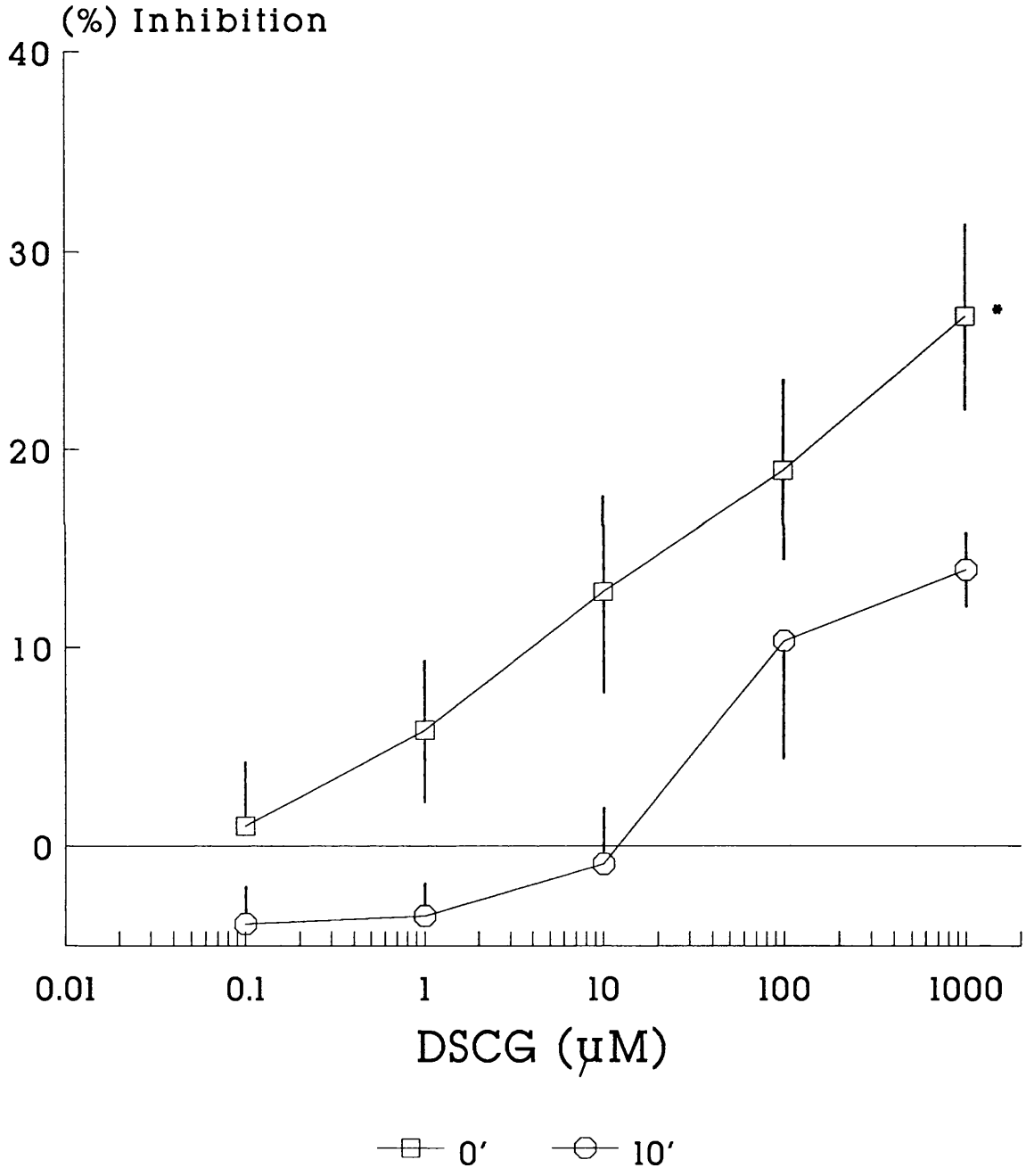


Fig 7.3 Effect of disodium cromoglycate (DSCG) on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 14.7±3.1 % (0') and 14.9±2.2 % (10') respectively, n=6, paired data.

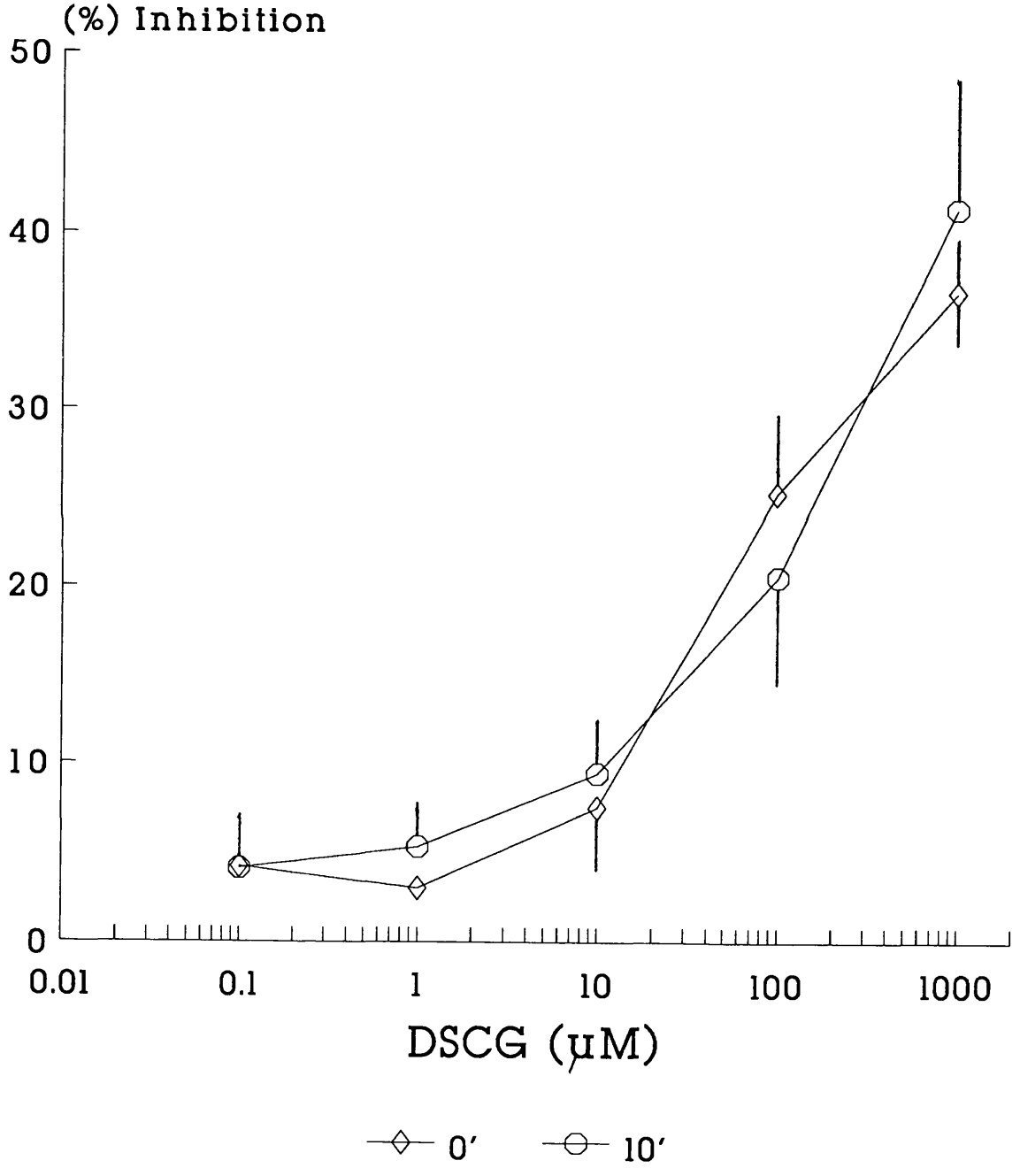


Fig 7.4 Effect of disodium cromoglycate (DSCG) on anti-IgE-induced histamine release from isolated human colonic submucosal/muscle mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 22.4±5.8 % (0') and 19.7±6.1 % (10') respectively, n=7, paired data.

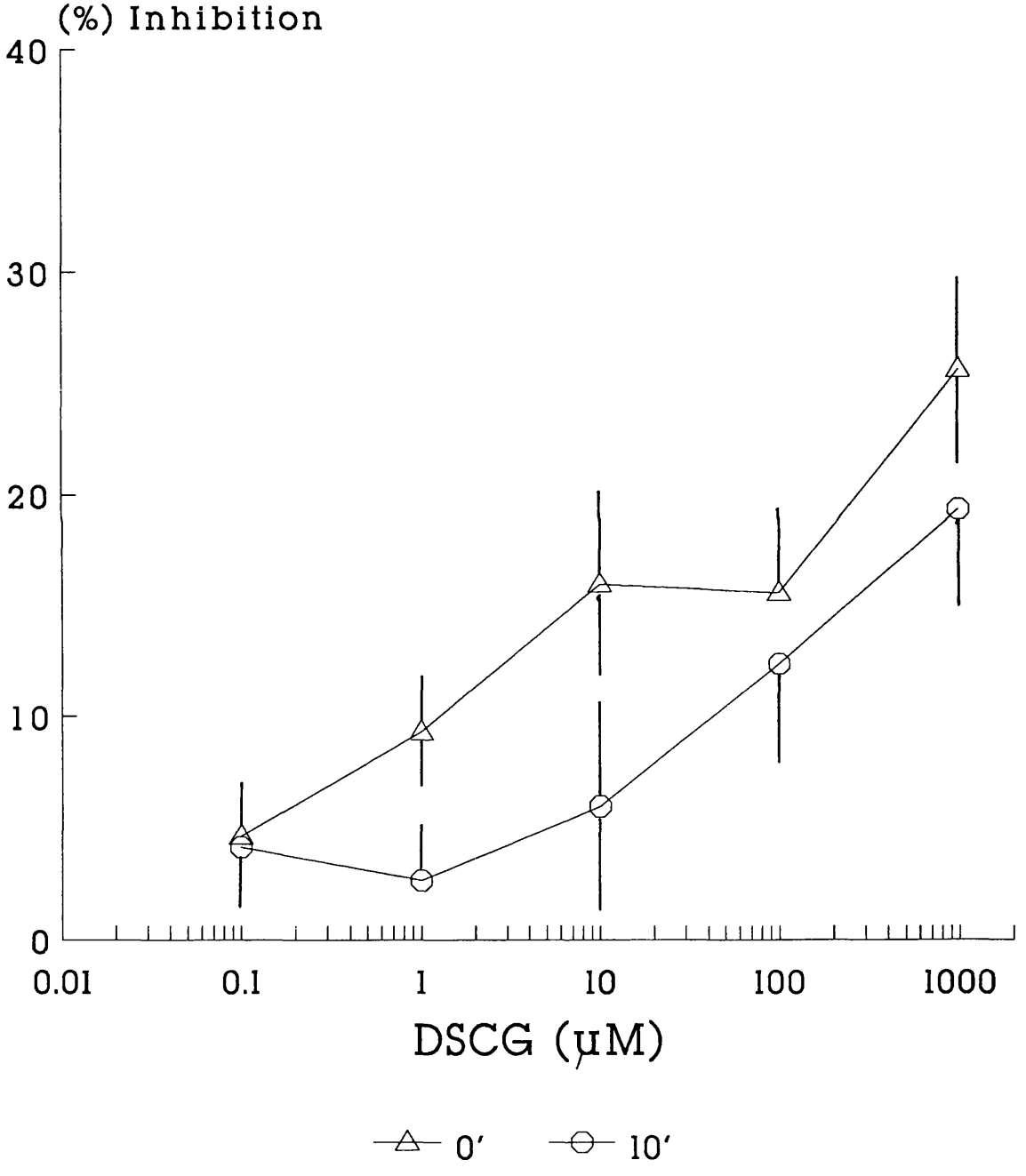


Fig 7.5 Effect of nedocromil sodium on anti-IgE-induced histamine release from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS). The drug was added simultaneously with stimulus to cells. The control releases were 20.9±5.3 %, n=4, HL; 12.1±0.7 %, n=6, HCM and 16.9±1.2 %, n=4, HCS.

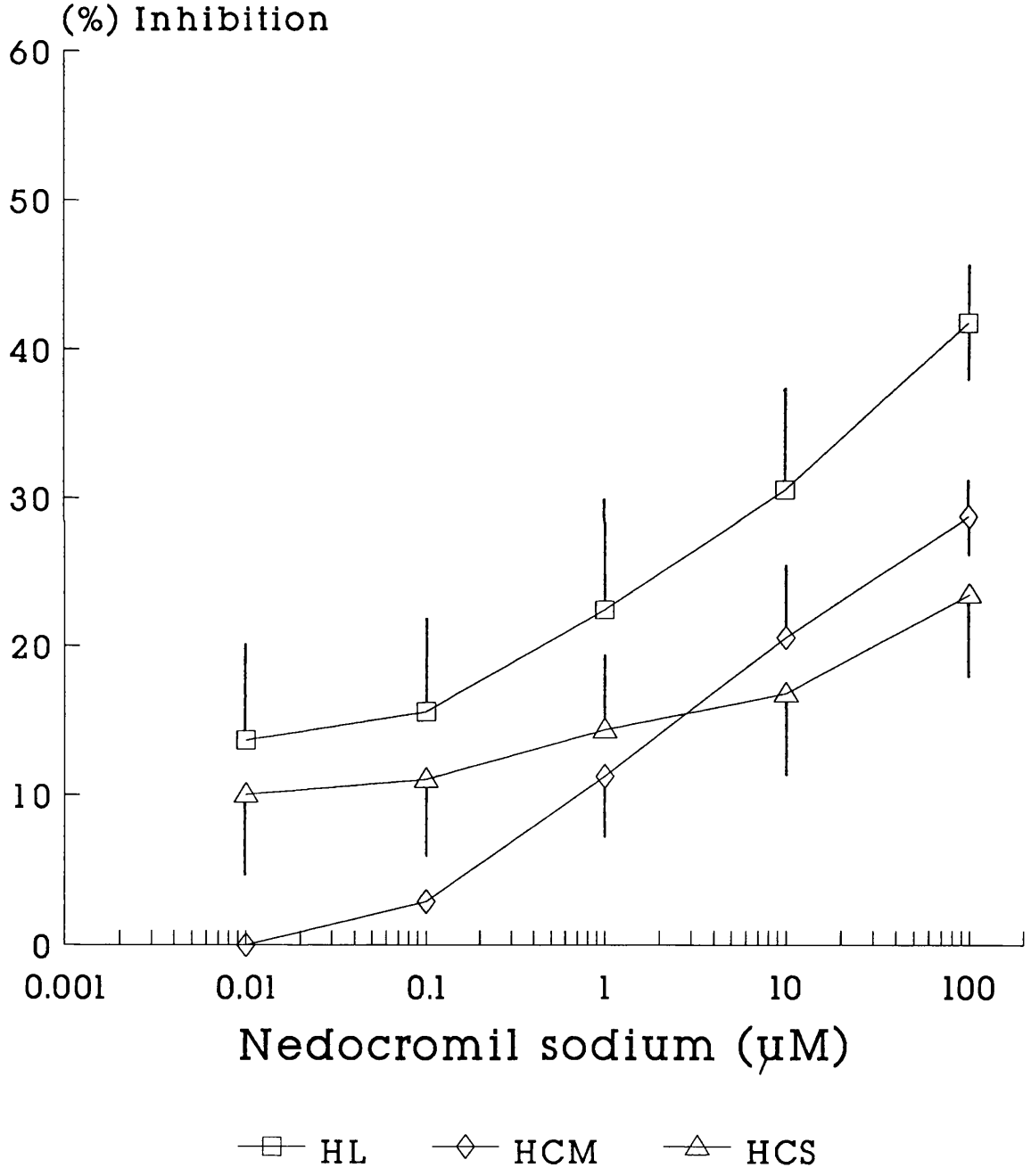


Fig 7.6 Effect of nedocromil sodium on anti-IgE-induced histamine release from isolated human lung mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 20.9±5.3 % (0') and 21.5±5.7 % (10') respectively, n=4, paired data.

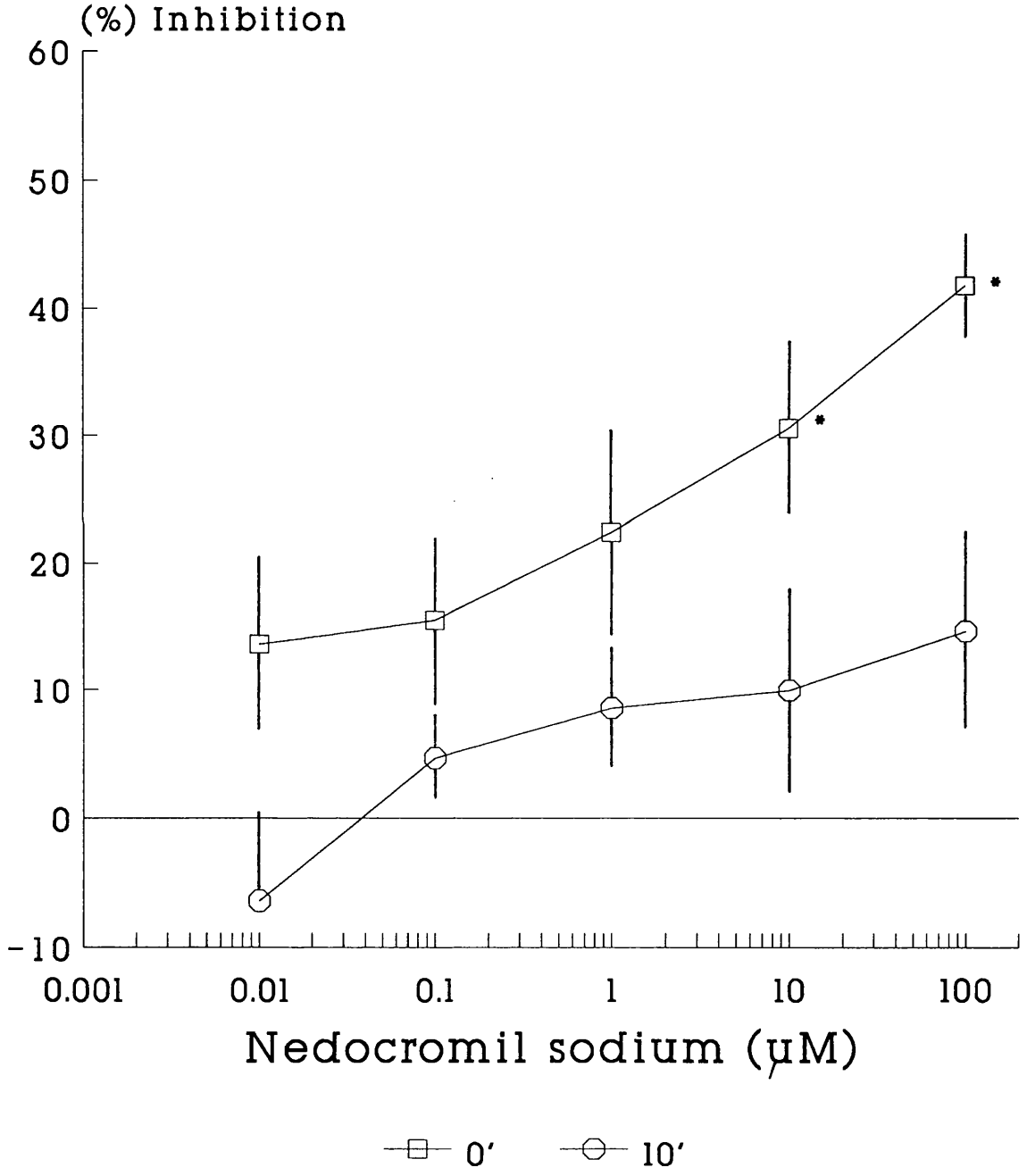


Fig 7.7 Effect of nedocromil sodium on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 12.1±0.7 % (0') and 13.1±1.2 % (10') respectively, n=6, paired data.

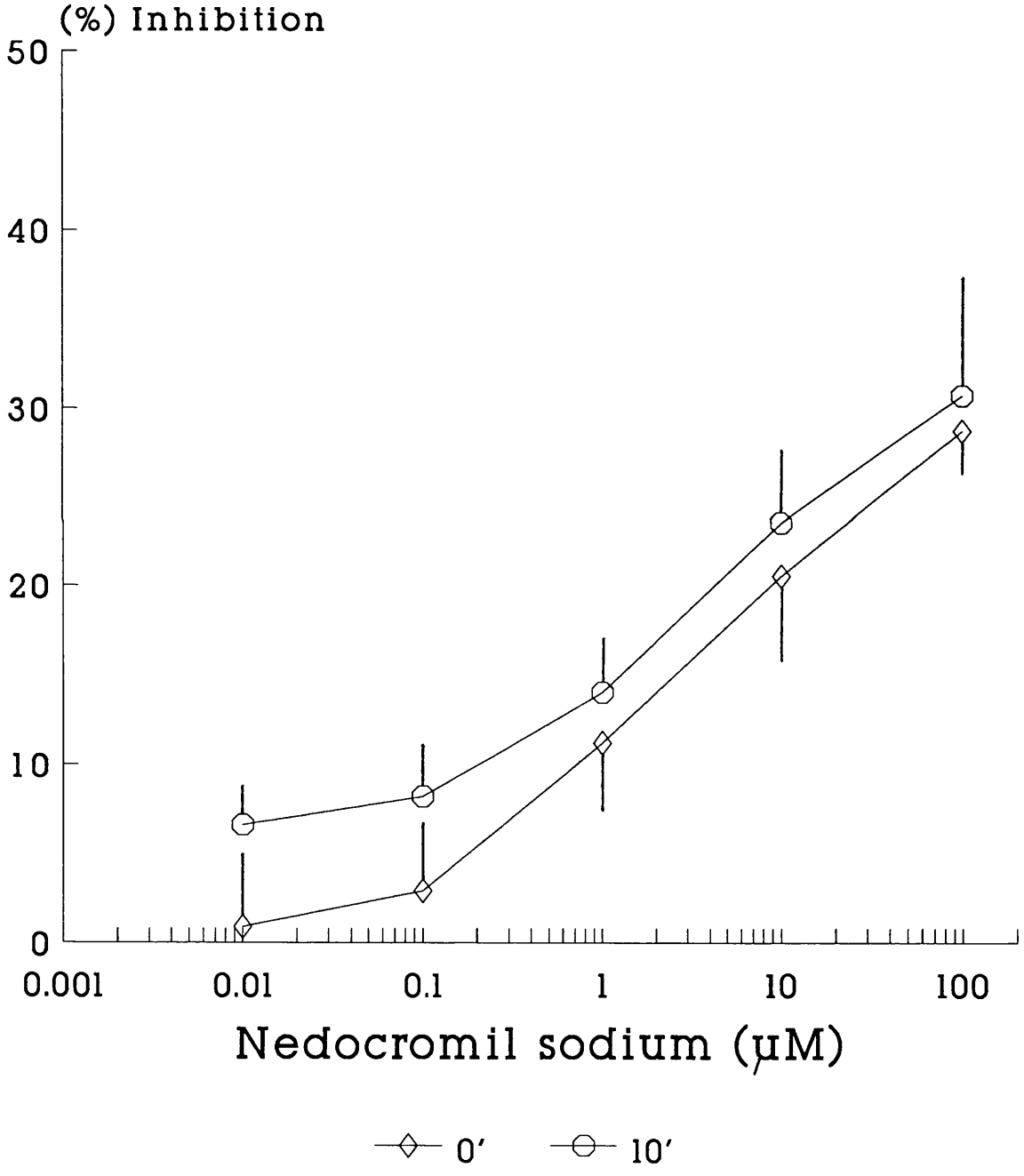


Fig 7.8 Effect of nedocromil sodium on anti-IgE-induced histamine release from isolated human colonic submucosal/muscle mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 16.9±1.2 % (0') and 15.4±1.9 % (10') respectively, n=4, paired data.

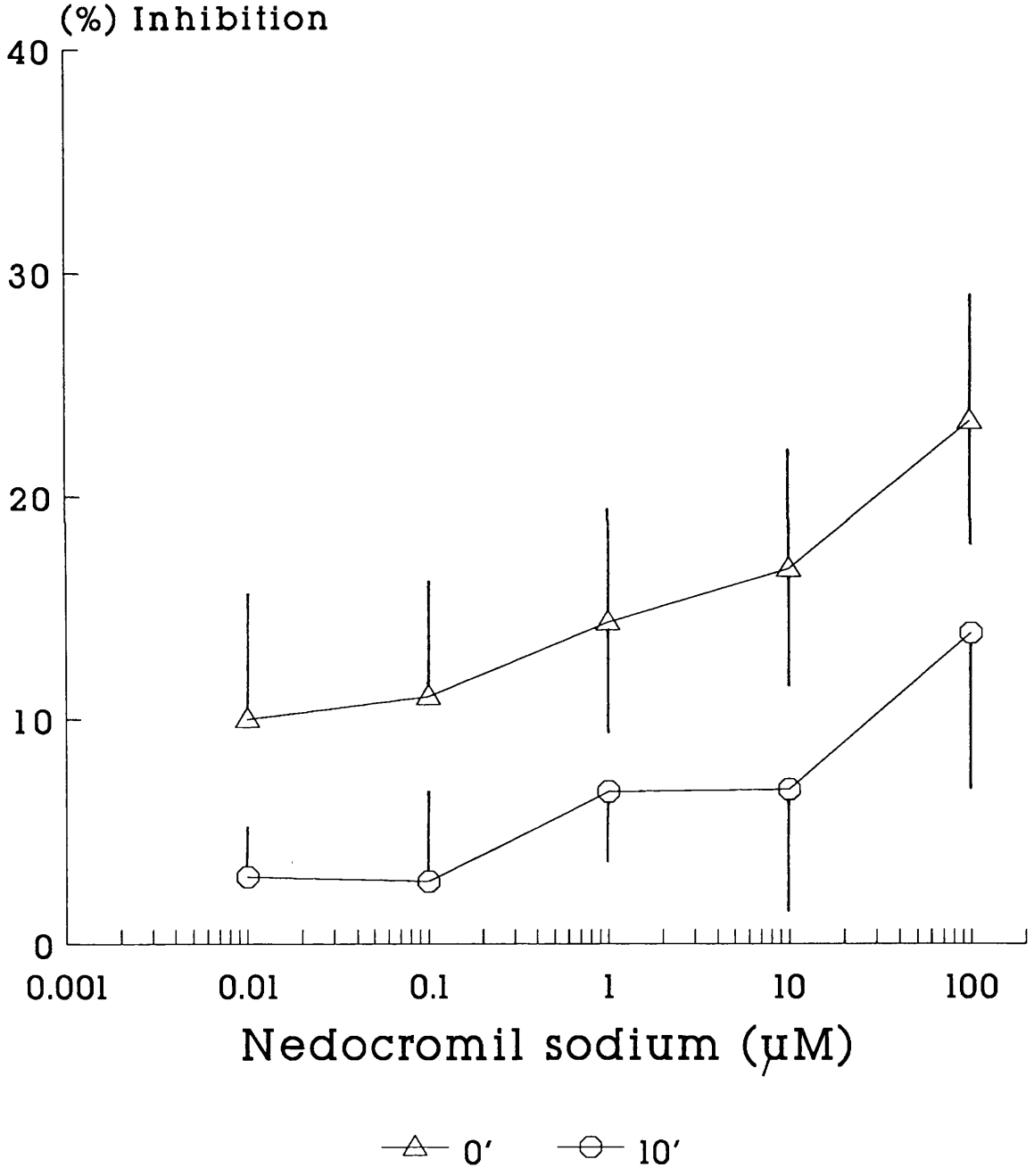


Fig 7.9 Effect of quercetin on anti-IgE-induced histamine release from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS). The drug was preincubated with cells for 10 min before challenge. The control releases were 24.6±7.9 %, n=4, HL; 16.9±4.7 %, n=5, HCM and 17.4±3.4 %, n=4, HCS.

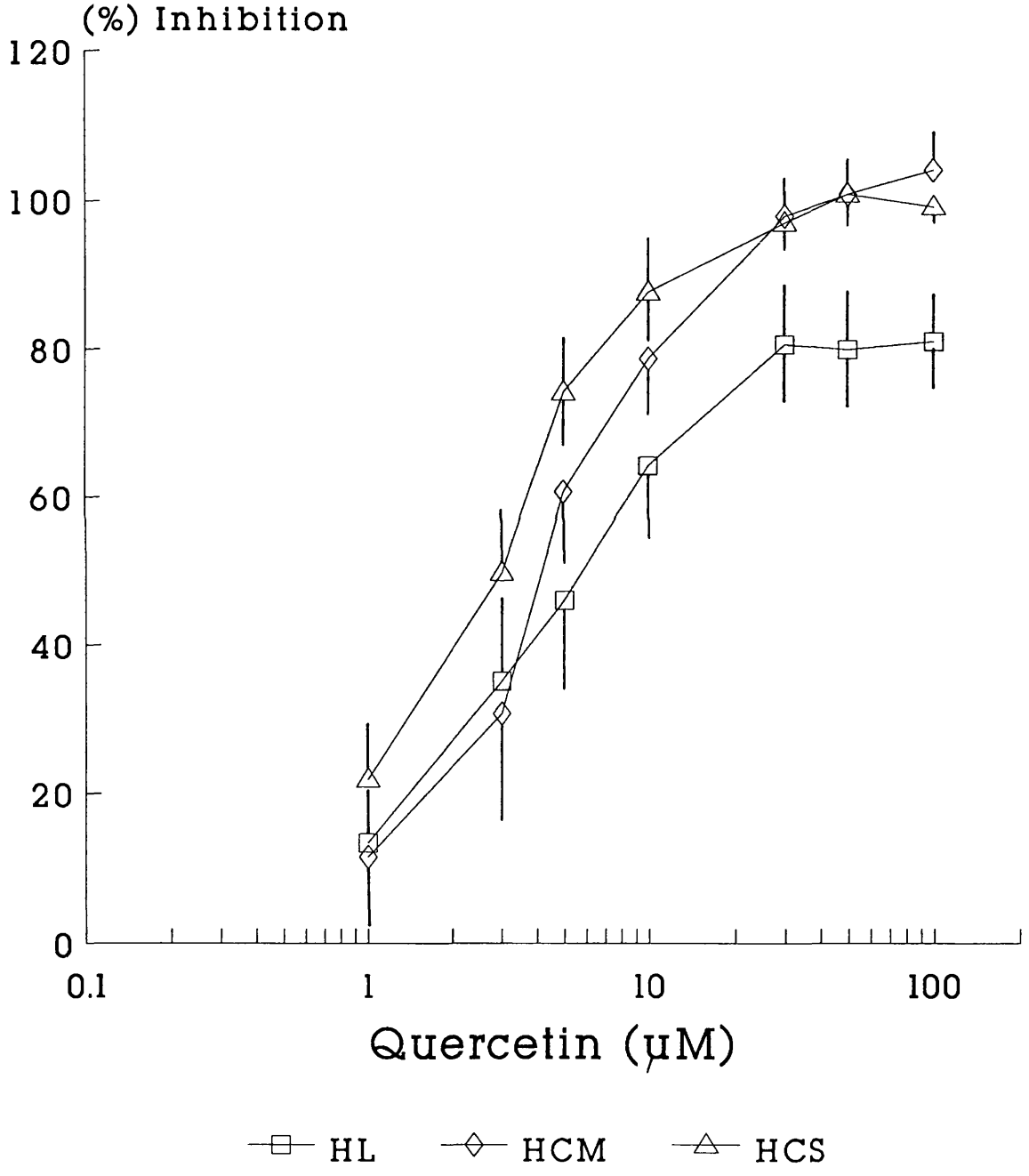


Fig 7.10 Effect of chrysin on anti-IgE-induced histamine release from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS). The drug was preincubated with cells for 10 min before challenge. The control releases were 24.6±7.9 %, n=4, HL; 16.9±4.7 %, n=5, HCM and 17.4±3.4 %, n=4, HCS.

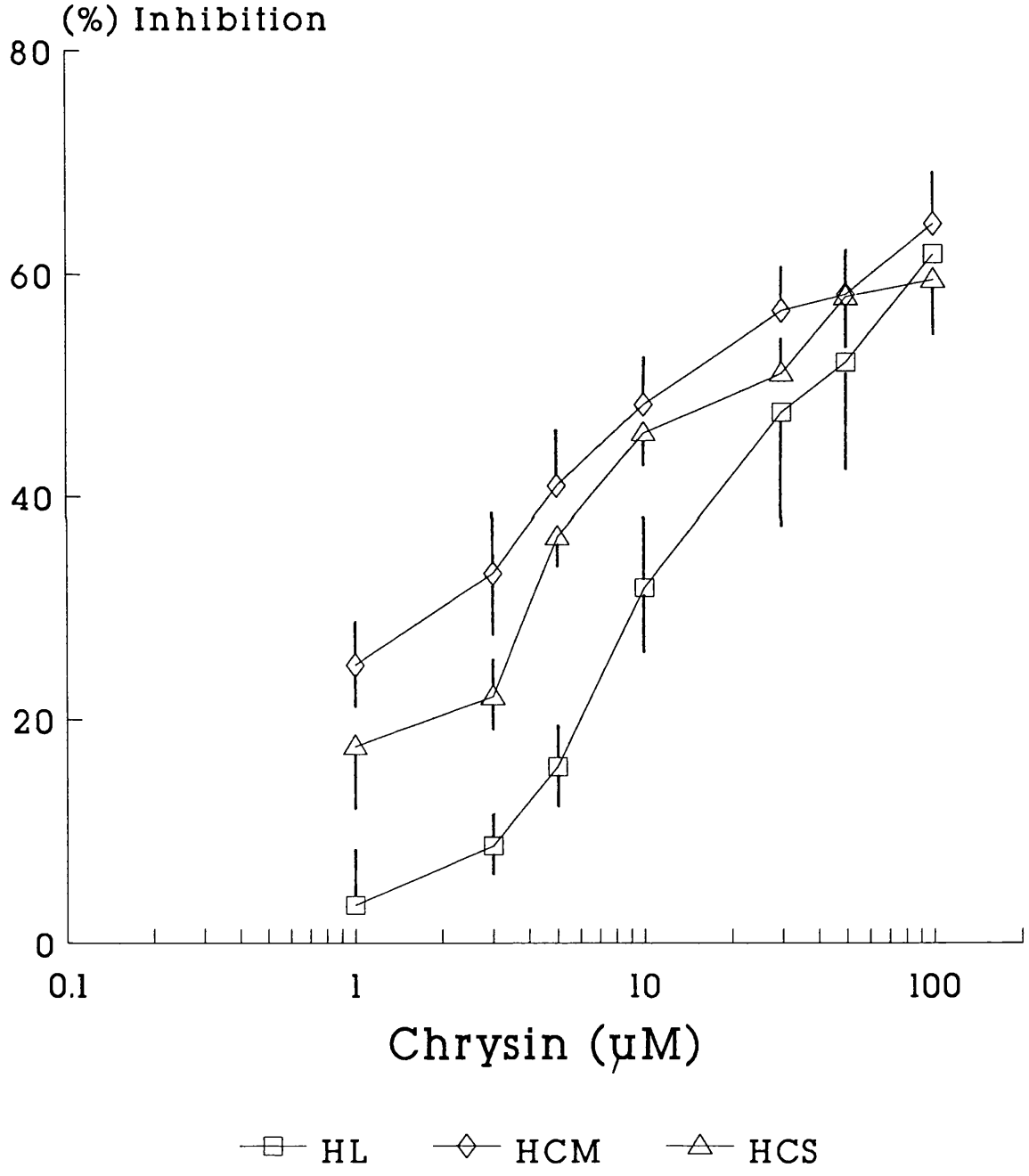


Fig 7.11 Effect of LU 48953 on anti-IgE-induced histamine release from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS). The drug was added simultaneously with stimulus to cells. The control releases were 28.1±3.9 %, n=4, HL; 14.7±3.1 %, n=6, HCM and 19.4±4.8 %, n=4, HCS.

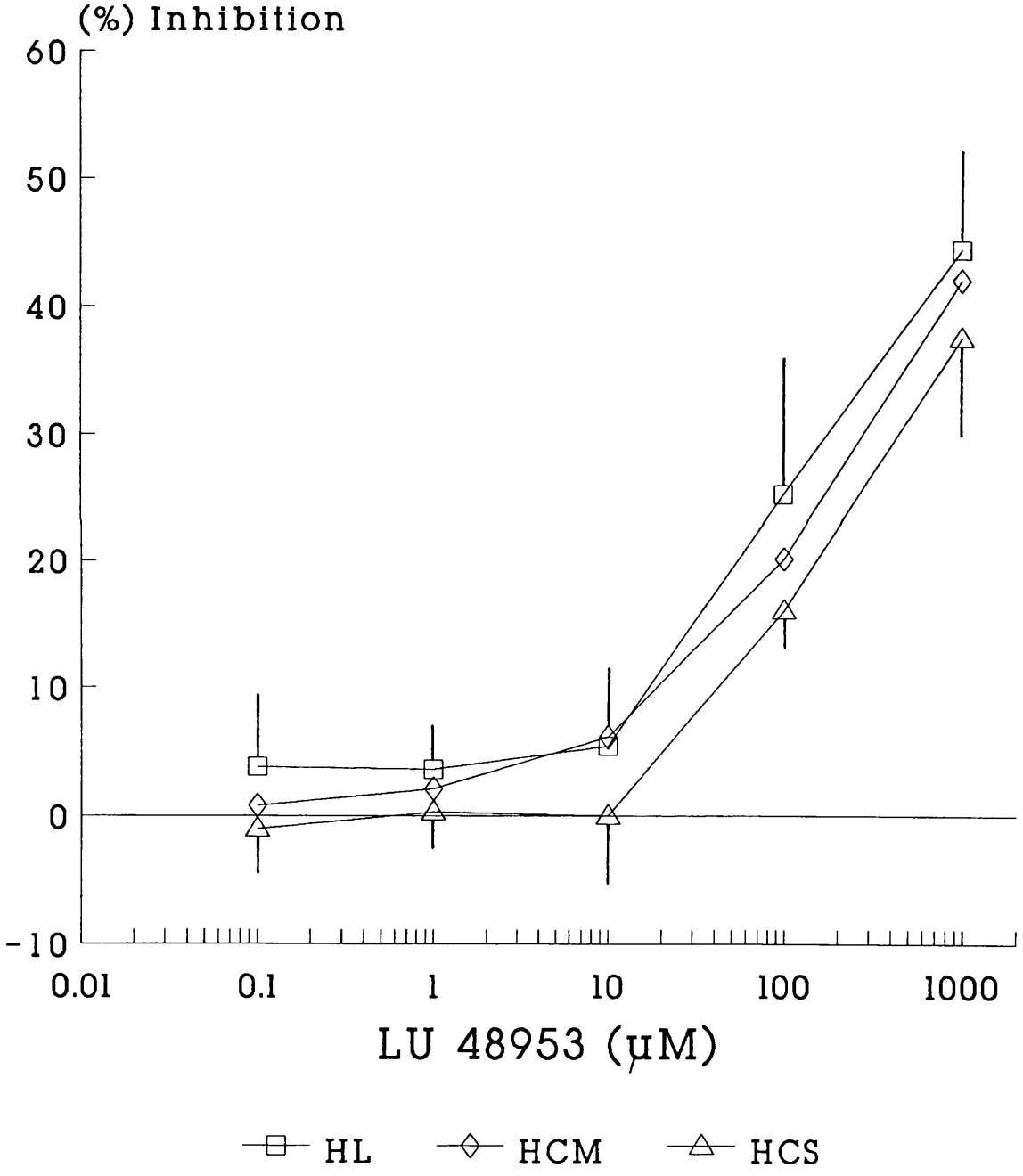


Fig 7.12 Effect of LU 48953 on anti-IgE-induced histamine release from isolated human lung mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 28.1±3.9 % (0') and 27.5±3.7 % (10') respectively, n=4, paired data.

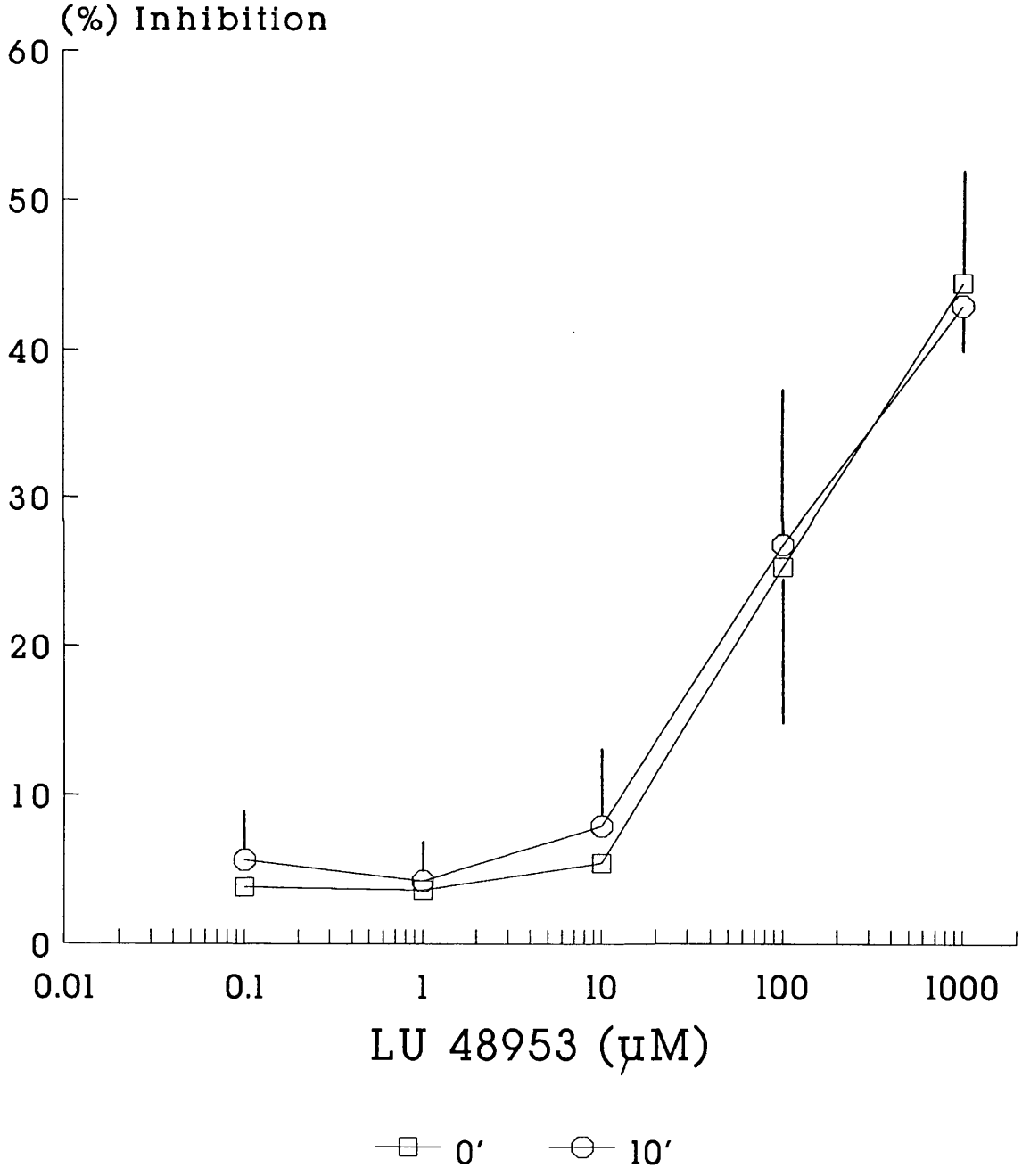


Fig 7.13 Effect of LU 48953 on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 14.7±3.1 % (0') and 14.9±2.2 % (10') respectively, n=6, paired data.

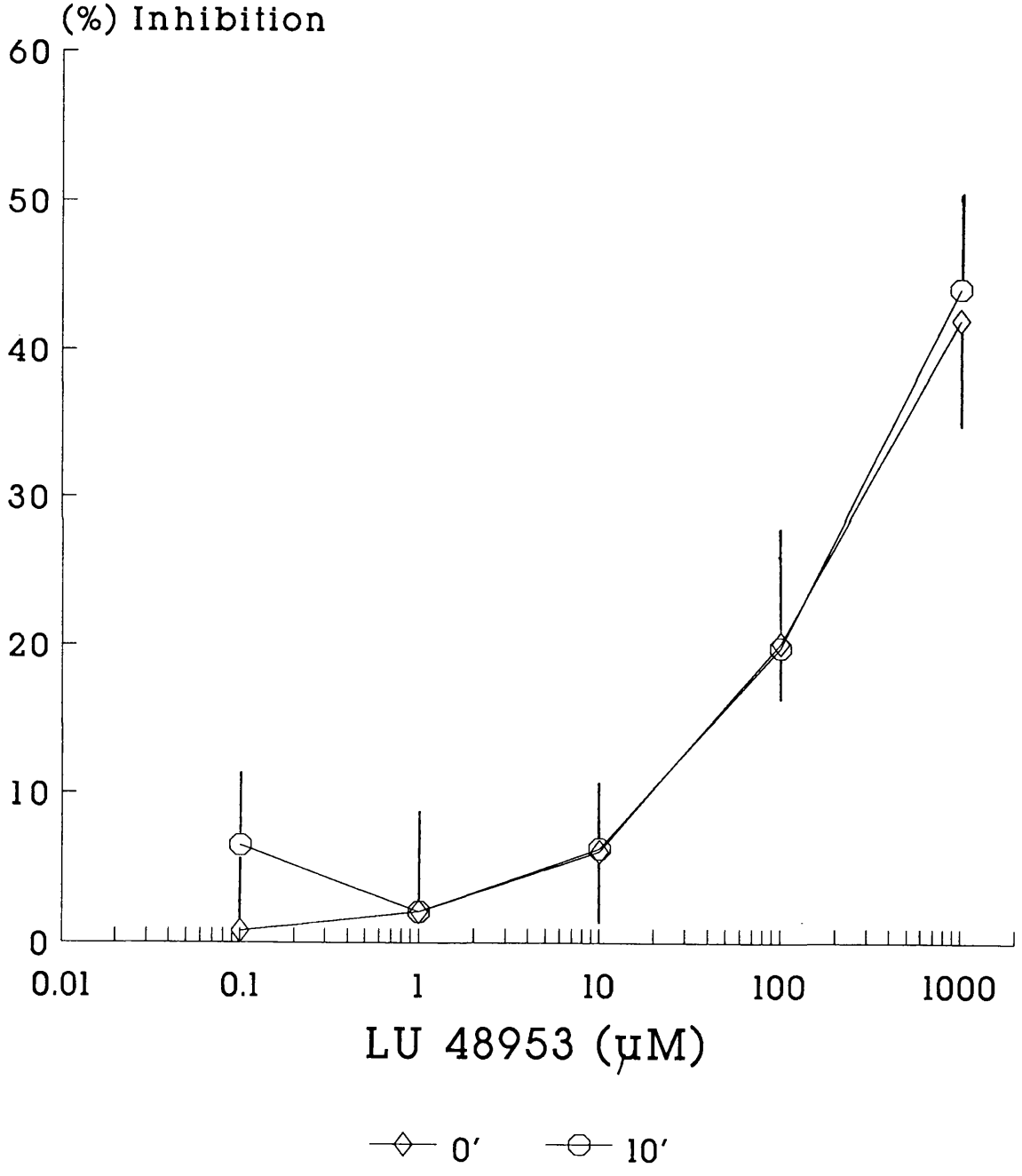


Fig 7.14 Effect of LU 48953 on anti-IgE-induced histamine release from isolated human colonic submucosal/muscle mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 19.4±4.8 % (0') and 16.8±3.1 % (10') respectively, n=4, paired data.

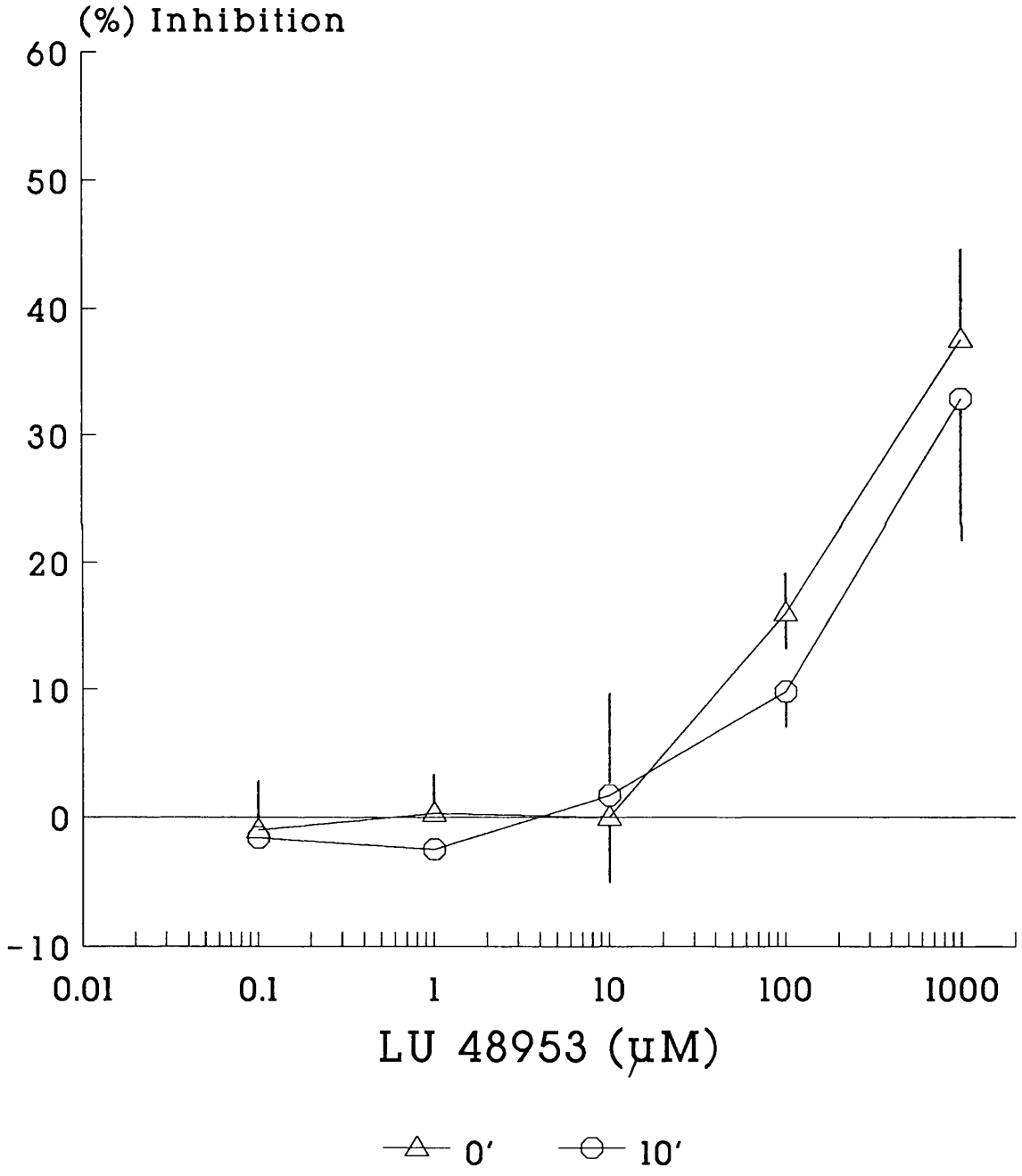


Fig 7.15 Effect of Bu_2cAMP on anti-IgE-induced histamine release from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS). The drug was preincubated with cells for 30 min before challenge. The control releases were 23.6±7.1 %, n=4, HL; 18.1±2.0 %, n=4, HCM and 17.2±1.3 %, n=4, HCS.

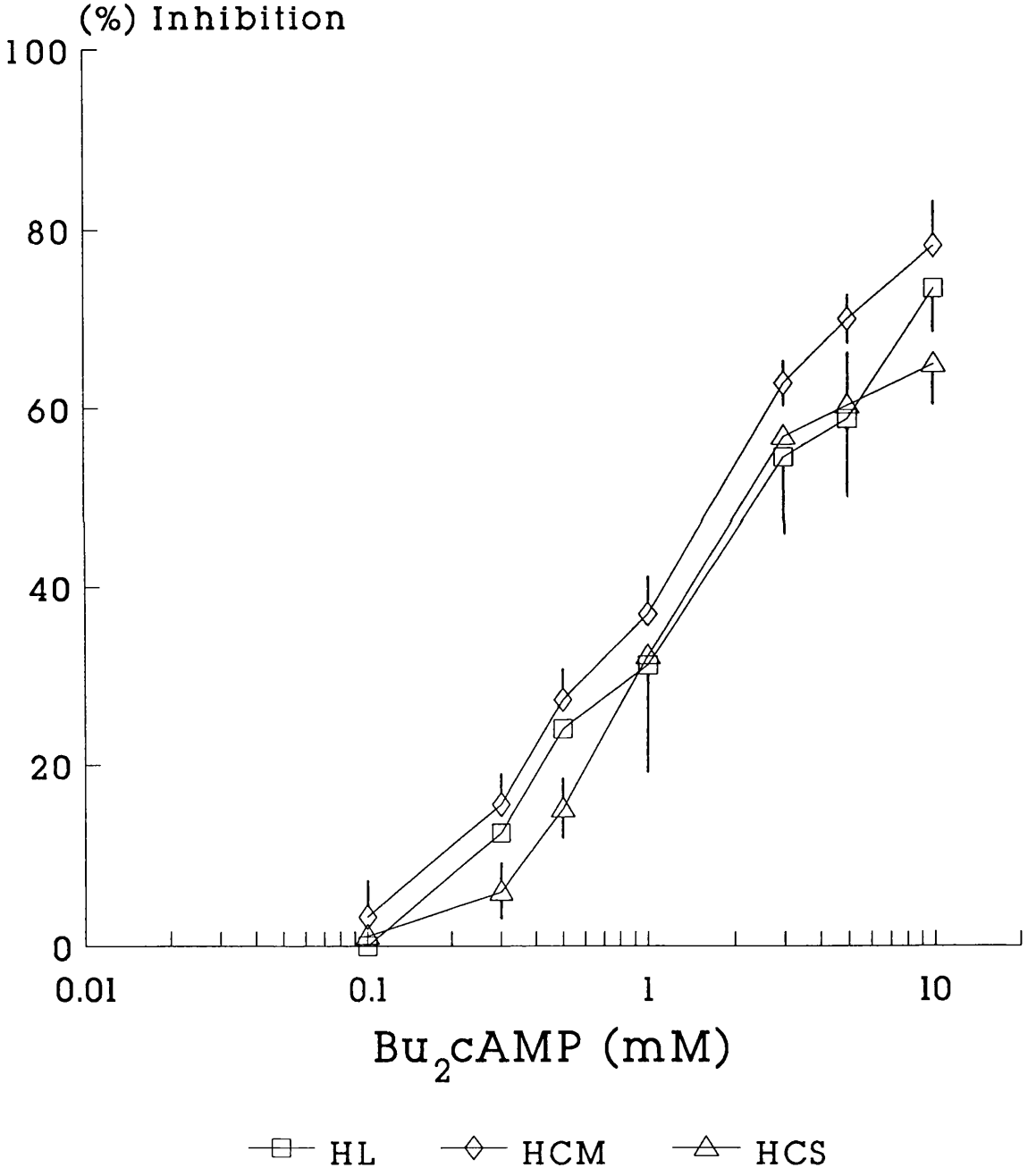


Fig 7.16 Effect of theophylline on anti-IgE-induced histamine release from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS). The drug was preincubated with cells for 10 min before challenge. The control releases were 29.8±4.5 %, n=4, HL; 17.2±4.3 %, n=4, HCM and 15.4±1.9 %, n=4, HCS.

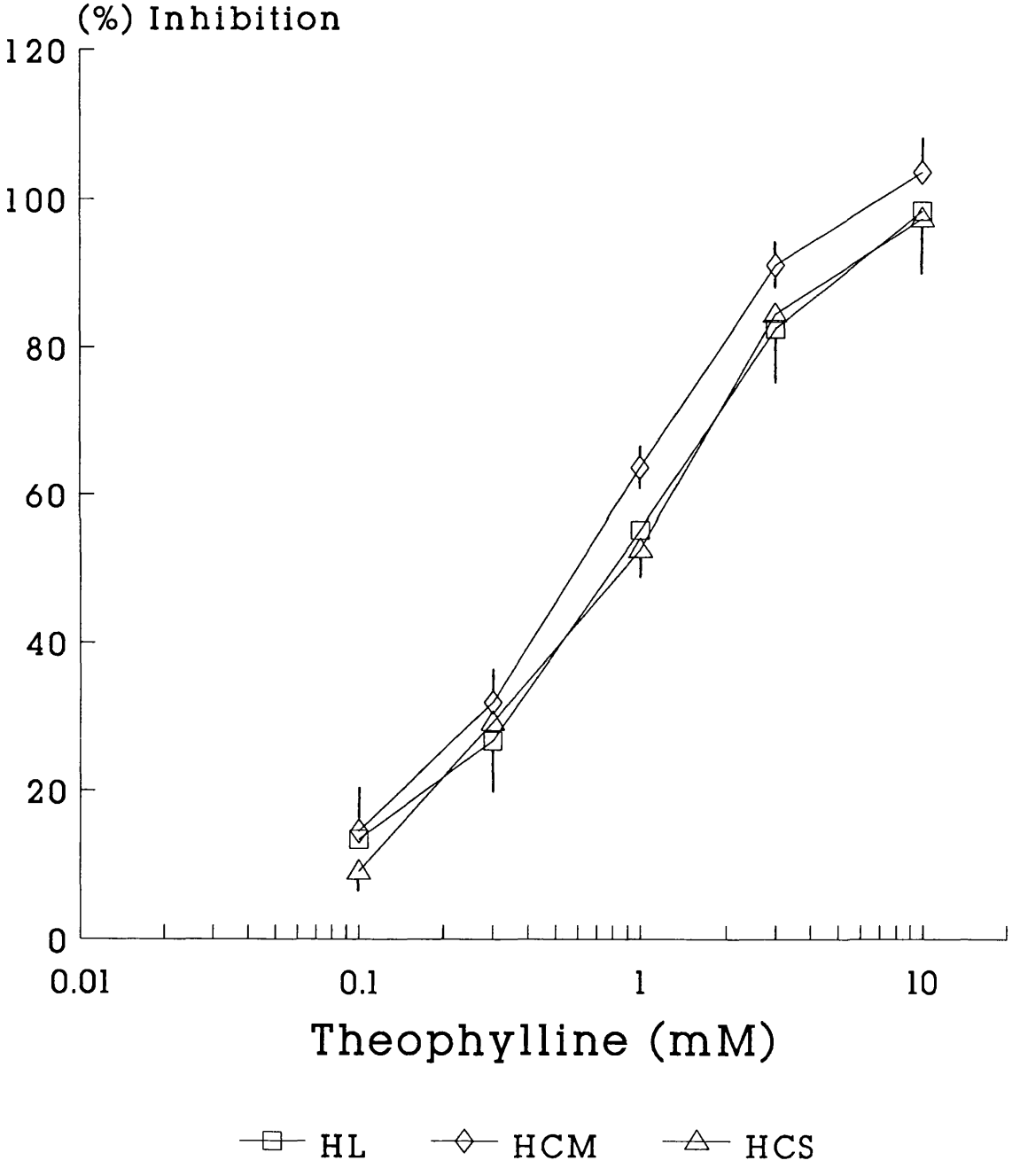


Fig 7.17 Effect of IBMX on anti-IgE-induced histamine release from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS). The drug was preincubated with cells for 10 min before challenge. The control releases were 23.6±7.0, n=5, HL; 20.0±5.2 %, n=4, HCM and 17.4±3.4 %, n=4, HCS.

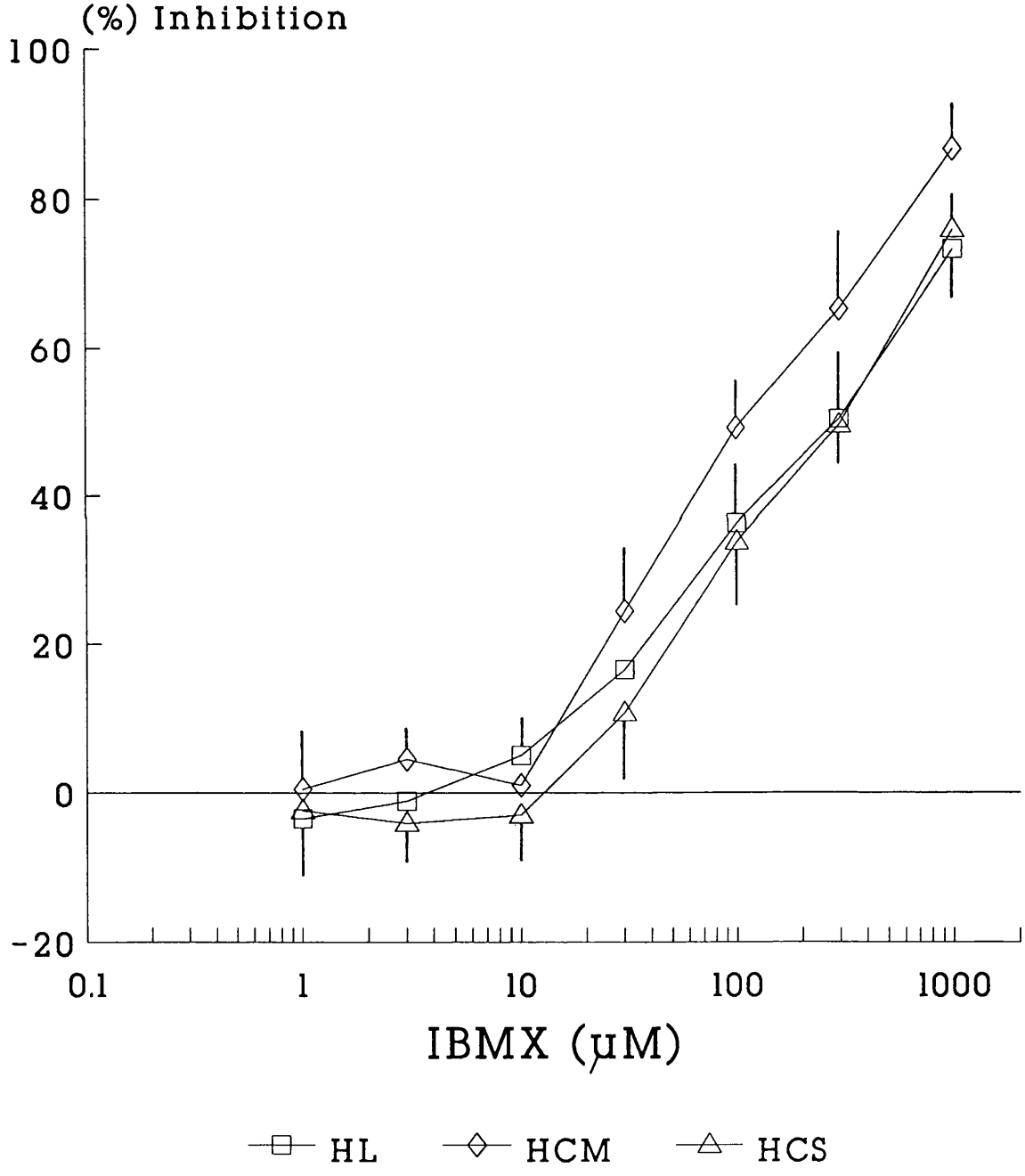


Fig 7.18 Effect of isoprenaline on anti-IgE-induced histamine release from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS). The drug was preincubated with cells for 10 min before challenge. The control releases were 29.8±4.5 %, n=4, HL; 17.4±3.7 %, n=5-6, HCM and 15.4±1.9 %, n=4, HCS.

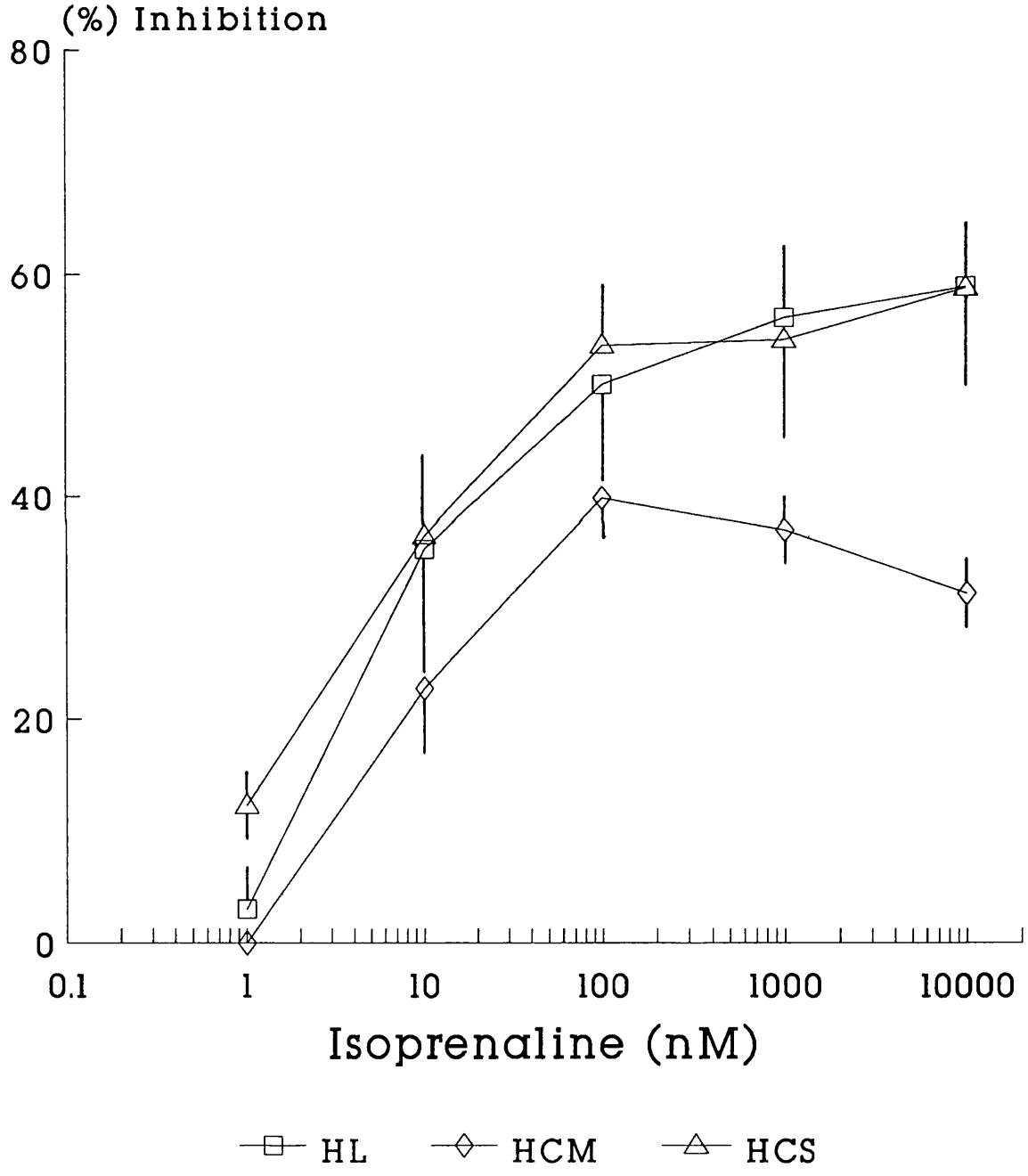


Fig 7.19 Effect of salbutamol on anti-IgE-induced histamine release from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS). The drug was preincubated with cells for 10 min before challenge. The control releases were 26.1±5.7 %, n=4, HL; 17.6±3.7 %, n=4-6, HCM and 18.4±2.4 %, n=4, HCS.

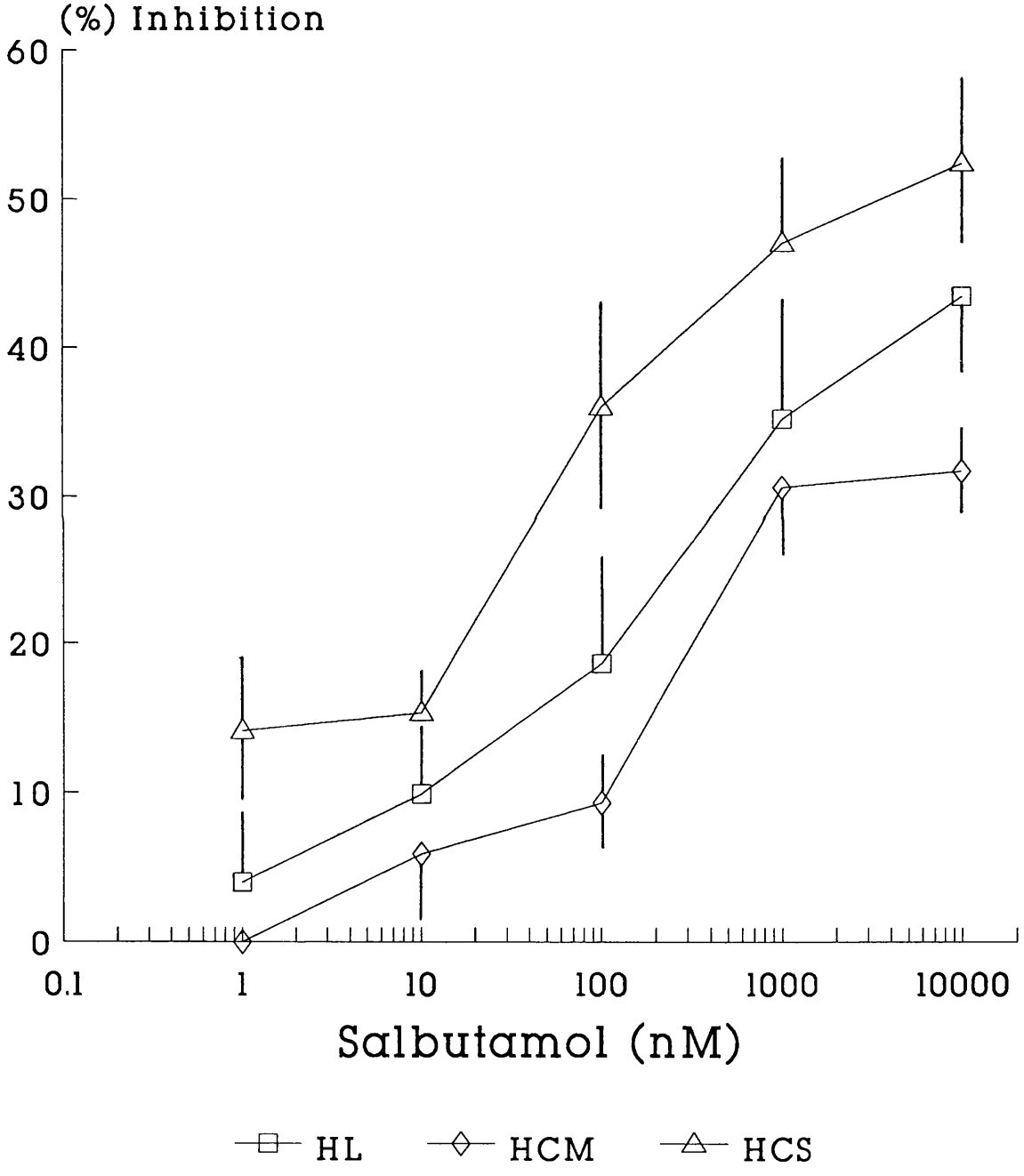


Fig 7.20 Effect of cimetidine on anti-IgE-induced histamine release from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS). The drug was preincubated with cells for 10 min before challenge. The control releases were 23.2±6.3 %, n=4-5, HL; 18.6±4.2 %, n=5, HCM and 17.4±3.4 %, n=4, HCS.

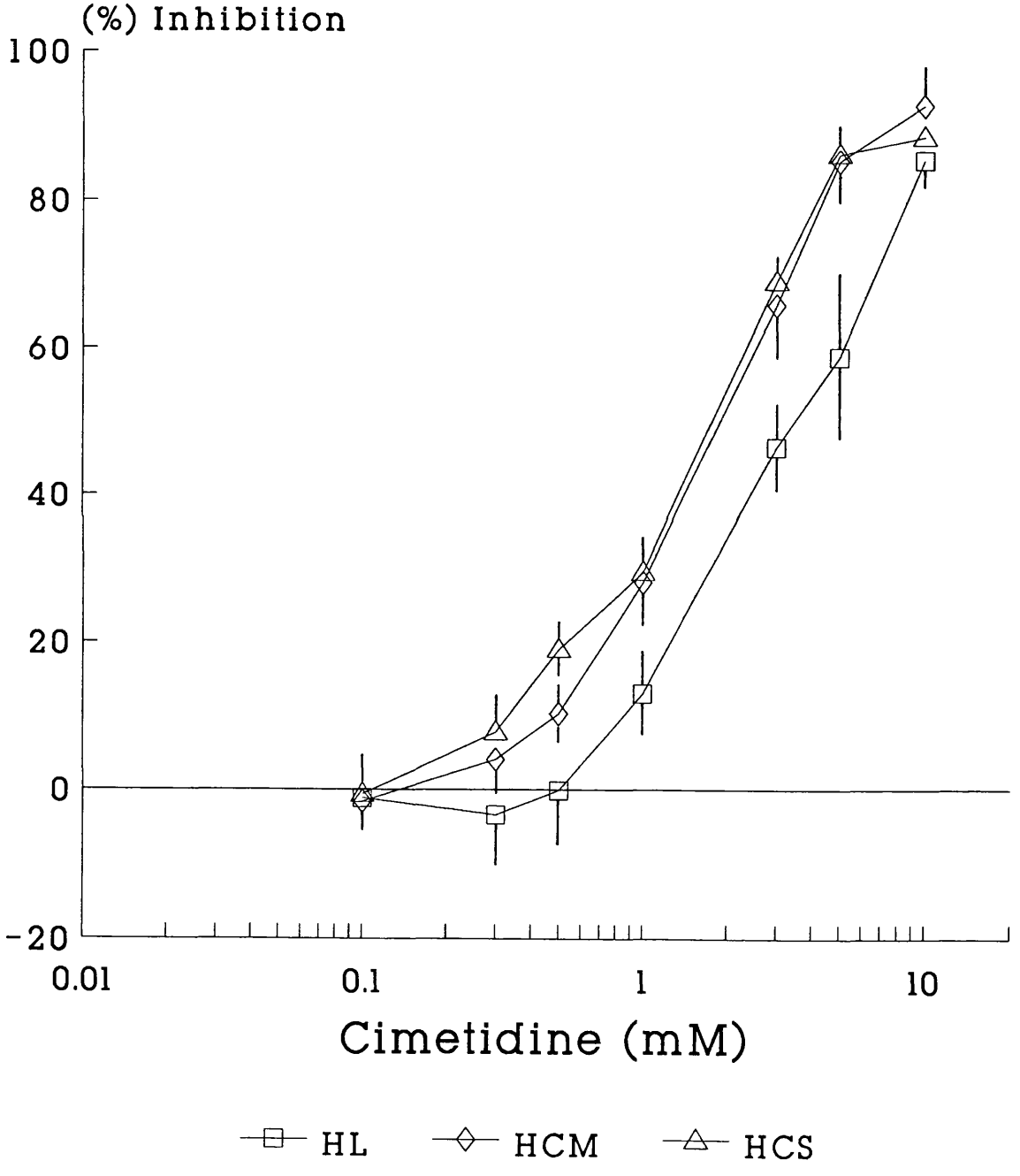


Fig 7.21 Effect of ranitidine on anti-IgE-induced histamine release from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS). The drug was preincubated with cells for 10 min before challenge. The control releases were 23.2±6.3 %, n=4-5, HL; 18.6±4.2 %, n=5, HCM and 17.4±3.4 %, n=4, HCS.

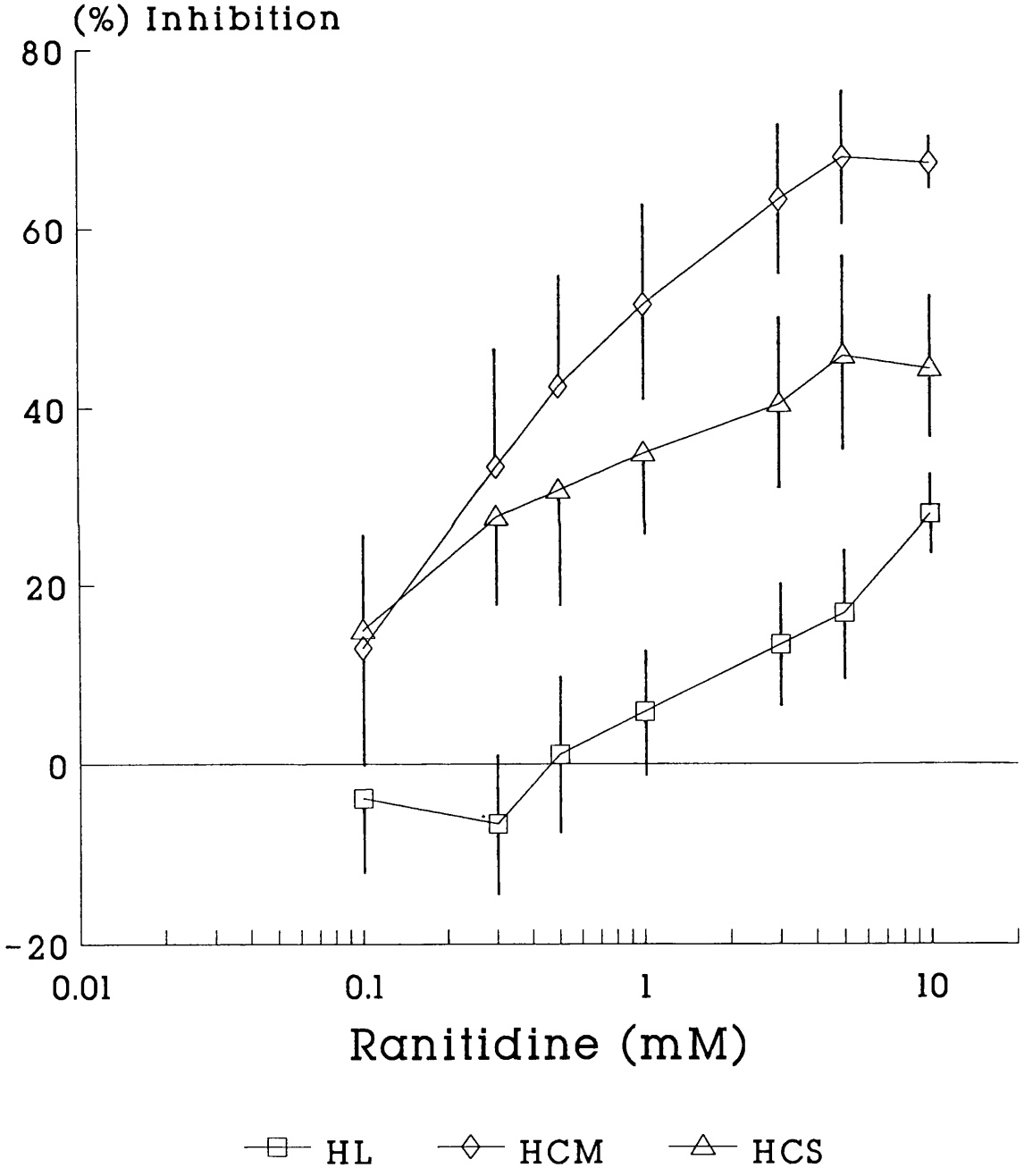


Fig 7.22 Effect of dimaprit on anti-IgE-induced histamine release from isolated human mast cells from the lung (HL), colonic mucosa (HCM) and colonic submucosa/muscle (HCS). The drug was preincubated with cells for 10 min before challenge. The control releases were 23.6±6.2 %, n=4, HL; 17.9±4.2 %, n=4, HCM and 16.6±2.8 %, n=4, HCS.

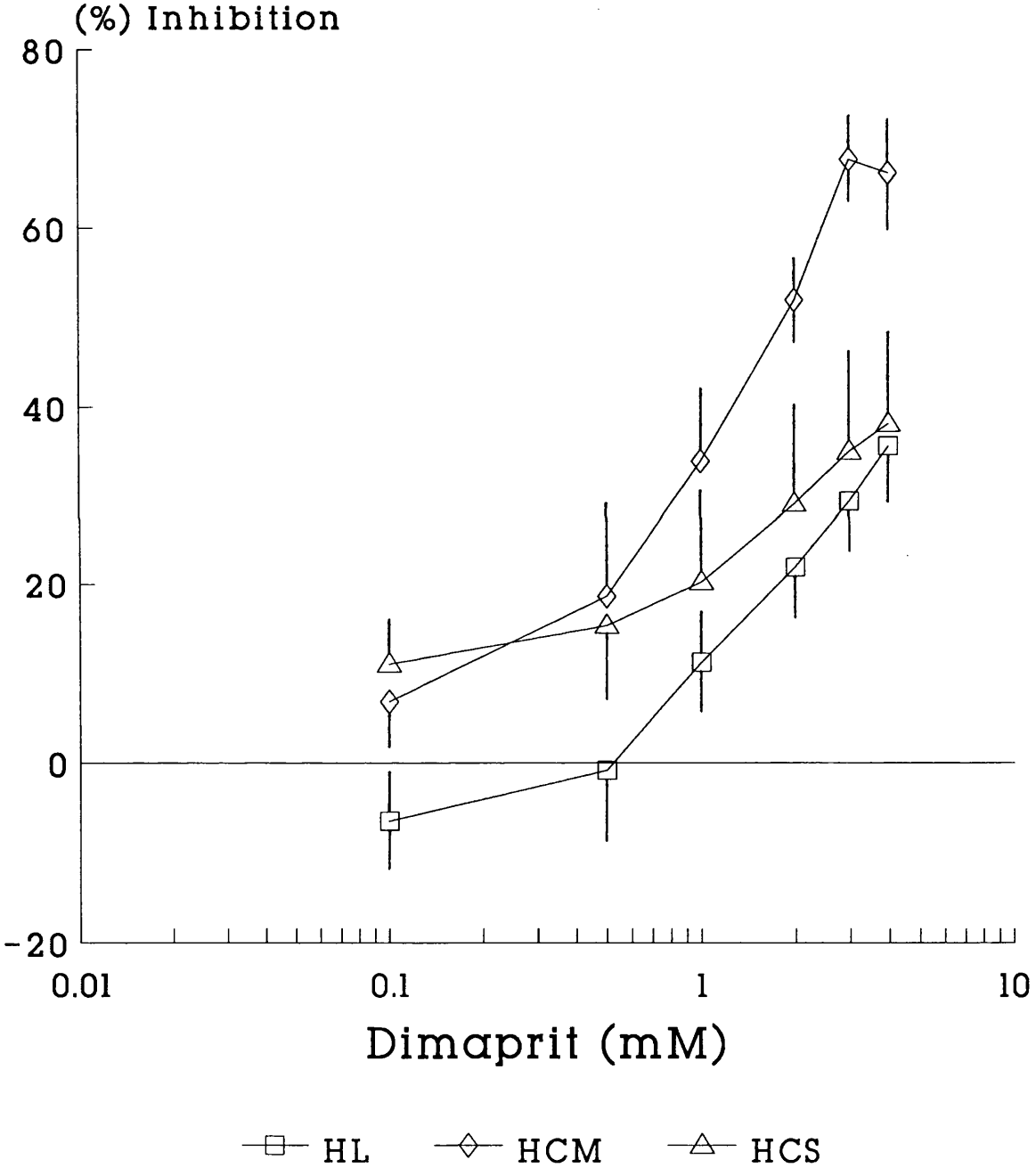


Table 7.1 Effect of MY 1250 on histamine release from human lung mast cells stimulated with anti-human IgE.

Concentration (μM)	(%) Inhibition NO PREINCUBATION n=4	10 MIN PREINCUBATION n=4
100	5.2±1.9	12.0±3.0
10	-0.8±1.4	5.0±2.2
1	-1.5±1.6	2.2±2.0
0.1	-2.2±1.4	0.7±0.8
0.01	-2.1±0.8	-0.1±0.6
Control release (%)	28.1±3.9	27.5±3.7

Table 7.2 Effect of MY 1250 on histamine release from human colonic mucosal mast cells stimulated with anti-human IgE.

Concentration (μM)	(%) Inhibition NO PREINCUBATION n=6	10 MIN PREINCUBATION n=6
100	9.0±3.1	20.6±5.1
10	3.0±1.8	7.2±3.8
1	1.3±3.5	6.7±5.5
0.1	-0.8±2.5	8.5±6.0
0.01	5.1±4.0	6.6±5.5
Control release (%)	15.7±2.8	15.8±1.9

Table 7.3 Effect of MY 1250 on histamine release from human colonic submucosal/muscle mast cells stimulated with anti-human IgE.

Concentration (μM)	(%) Inhibition NO PREINCUBATION n=4	10 MIN PREINCUBATION n=4
100	3.1±1.8	7.1±3.4
10	1.3±3.8	0.5±1.9
1	-0.5±2.3	-2.2±3.1
0.1	-2.0±3.6	-3.5±3.8
0.01	-3.1±3.2	-3.7±1.4
Control release (%)	20.8±4.6	18.1±2.8

CHAPTER EIGHT

SOME STUDIES ON HUMAN COLONIC MUCOSAL MAST CELLS FROM PATIENTS WITH CROHN'S DISEASE AND ULCERATIVE COLITIS

8.1 Introduction

Inflammatory bowel disease (IBD) is a collective term used to describe two chronic inflammatory disorders affecting the human large and small intestine; namely Crohn's disease and ulcerative colitis. Both conditions are characterized clinically by abdominal discomfort, diarrhoea, weight loss and fever, and histologically by a chronic inflammatory infiltrate in the mucosa of affected areas. The aetiology of IBD is unknown and the involvement of the mast cell in these conditions has been the subject of many contrasting reports.

In general, an increase in the mast cell population of the colonic and rectal mucosa has been described in patients suffering from ulcerative colitis [28,386]. Moreover, the potential role of this cell type in this intestinal disorder has been further implicated by the clinical usefulness of the anti-allergic compound DSCG [387-389]. In contrast, there are conflicting results in the literature concerning the numbers of mast cells found in patients suffering from Crohn's disease. Thus, it has been reported that the cell density was increased [28,390], unchanged [391] or even decreased [386] during active phases of this disorder. However, it must be noted that these studies were performed before it was widely recognised that human intestinal mast cells were not well preserved in formaldehyde-based fixatives and required special fixation and staining techniques for their presence to be demonstrated [259,260]. With this in mind Sanderson et al [392], in a more recent study, have reported a reduction in mast cell numbers in inflamed ileal biopsies fixed in Carnoy's solution from children with Crohn's disease.

In an in vitro study, Fox et al [393] have shown that, upon immunological

stimulation, enzymically dispersed mast cells from inflamed intestinal mucosa of patients with IBD released a greater quantity of histamine, PGD_2 and LTC_4 when compared to those derived from normal tissues of the same patients. This finding possibly indicates an increased level of mast cell activation in areas of the intestine affected by the disease. With this in mind, it is the aim of the present study to extend further the investigation of Fox et al [393]. Enzymically dispersed colonic mucosal mast cells from patients suffering from Crohn's disease and ulcerative colitis were examined and compared with those cells derived from normal tissues of cancer patients in terms of their basic histochemical and functional properties.

8.2 Methods

All methods used in this study were as described in chapter two.

8.3 Results

Unless otherwise stated, histamine release in experiments involving anti-allergic compounds were induced by anti-human IgE.

8.3.1 Basic properties of colonic mucosal mast cells from control patients (CR) and patients with Crohn's disease (CD) and ulcerative colitis (UC).

The enzymic dispersion procedure was significantly more effective in isolating colonic mucosal mast cells from patients with Crohn's disease ($P < 0.001$) and ulcerative colitis ($P < 0.001$) as compared to the control group (table 8.1). This technique recovered $0.9\pm0.1 \times 10^6$ (CR), $2.1\pm0.4 \times 10^6$ (CD, $P < 0.001$) and $2.2\pm0.6 \times 10^6$ (UC, $P < 0.001$) mast cells per gram wet tissue (n=4-10). Mast cells comprised 6.2±1.4 (CR), 11.0±3.3 (CD) and 7.8±1.8 % (UC) of the total nucleated cells (n=4-10). Taking this and the dispersion efficiency into account, an estimation of the total number of mast cells per gram wet tissue in these three groups was as follows: $5.4\pm1.0 \times 10^6$ (CR), $6.8\pm0.8 \times 10^6$ (CD, $P < 0.001$) and $7.1\pm0.5 \times 10^6$ (UC, $P < 0.01$). However, despite the significant variations in total mast cell numbers, the estimated histamine content (μg/g wet tissue) was not statistically different for the three groups of patients. This

observation was probably due to the significantly lower histamine content per mast cell of IBD patients ($P < 0.001$ in both cases) as compared to controls.

Cells obtained from the three groups of patients were structurally intact as judged under light microscopy, highly viable and exhibited low spontaneous histamine release (table 8.1). Isolated colonic mucosal mast cells from these cell preparations were predominantly sensitive to formaldehyde fixation although those derived from Crohn's and colitic subjects contained a significantly higher percentage of the formaldehyde insensitive subtype; 8.7±1.3 (CR), 20.0±2.7 (CD, $P < 0.001$) and 17.0±3.1 % (UC, $P < 0.001$) respectively. Finally, mast cells from both controls and IBD patients were exclusively alcian blue positive and did not counterstain with safranin.

8.3.2 Histamine release by IgE-directed ligands

Isolated colonic mucosal mast cells from control, Crohn's and colitic subjects released histamine in a dose-dependent fashion in response to stimulation with anti-human IgE (fig 8.1a) and the lectin concanavalin A (fig 8.1b). In each case, although not statistically significant, cells from the control group were generally the most reactive.

8.3.3 Histamine release by calcium ionophores and polybasic compounds

The calcium ionophores A23187 (fig 8.2a) and ionomycin (fig 8.2b) both produced a pronounced and comparable secretion of histamine from isolated colonic mucosal mast cells from the three groups of patients. In contrast, the polybasic agent compound 48/80 (table 8.2) and the neuropeptide substance P (table 8.3) both had essentially no effect against any of these cell preparations.

8.3.4 Effects of chromones

DSCG was significantly more effective in inhibiting anti-IgE-induced histamine release from human colonic mucosal mast cells from Crohn's patients as compared to those derived from controls (fig 8.3). The chromone, however, exhibited tachyphylaxis

against the former cells (fig 8.6) whereas this phenomenon was not observed with the latter cells (fig 8.5). In contrast, maximum inhibition obtained for colonic mucosal mast cells from subjects with ulcerative colitis was comparable to that observed for the control group (fig 8.4). Moreover, the inhibition achieved was unaltered upon preincubation (fig 8.7).

Nedocromil sodium, a congener of DSCG, also produced a dose-dependent inhibition of anti-IgE-induced histamine release from human colonic mucosal mast cells from the three groups of patients (figs 8.8 and 8.9). Although the average maximum inhibition achieved was similar in each case, the individual response of the two IBD groups was extremely variable; maximum inhibition ranged from 16-49 % for Crohn's patients and 12-72 % for colitic subjects. Similarly to DSCG, tachyphylaxis was observed for colonic mucosal mast cells from Crohn's patients (fig 8.11) but not from those of controls (fig 8.10) or colitic subjects (fig 8.12).

8.3.5 Effects of LU 48953 and MY 1250

LU 48953 suppressed the anti-IgE-induced histamine release from human colonic mucosal mast cells of control patients in a concentration-related manner (fig 8.13), and was equally active against these cells from the two IBD groups. In contrast to the chromones, LU 48953 exhibited no tachyphylaxis against any of the three mast cell preparations studied (figs 8.14-8.16).

MY 1250 was only active at the highest drug concentration tested (10^{-4} M). The weak inhibition observed, which was unaffected by preincubation (figs 8.18-8.20), was comparable for both controls and the two IBD groups (fig 8.17).

8.3.6 Effects of cAMP-active compounds

The phosphodiesterase inhibitor theophylline was a potent inhibitor of anti-IgE-induced histamine release from colonic mucosal mast cells from controls and patients suffering from Crohn's disease and ulcerative colitis (fig 8.21). These mast cells also responded to the β-adrenoceptor agonists isoprenaline (fig 8.22) and salbutamol (fig

8.23) but the effects observed were markedly less pronounced than with theophylline.

8.4 Discussion

The present study has demonstrated that functional human mast cells may be obtained by the enzymic dispersion of actively inflamed colonic mucosa from patients with Crohn's disease and ulcerative colitis. Moreover, these cells have been compared with those derived from macroscopically normal tissues of cancer patients (controls) in terms of their basic histochemical and functional properties.

The aetiology of both ulcerative colitis and Crohn's disease is unknown but a number of factors of genetic, environmental and dietary nature have been implicated in the expression of the two disorders [394]. The involvement of the intestinal mast cell in these inflammatory conditions is controversial and information has mainly been derived from studies on biopsies from actively inflamed tissues. In general, there is now some agreement concerning the increase in intestinal mast cell number found in patients suffering from ulcerative colitis [28,386]. In contrast, the situation with respect to Crohn's disease is far from clear cut. Hiatt and Katz [28] claimed that there was an increase in the mast cell density, Thompson and Buchanon [391] reported that there was no significant change, whereas Lloyd et al [386] observed a dramatic reduction. These studies, however, were performed before it was widely recognised that human intestinal mucosal mast cells were poorly preserved in formaldehyde-based fixatives [259,260]. With this in mind, Sanderson et al [392] studied intestinal biopsies fixed in Carnoy's solution from children with active Crohn's disease and reported a significant reduction in mast cell density in both the colon and terminal ileum. Moreover, these authors also observed a much lower histamine content in the inflamed biopsies as compared to those from normal patients. However, in two recent studies, and in contrast with the results of Sanderson et al [392], Raithel et al [395] and Schmidt et al [396] have reported a higher histamine content in inflamed tissues of subjects with Crohn's disease and ulcerative colitis . Moreover, the increase in amine content associated with the former disorder was observed to be accompanied by a reduction in intestinal diamine oxidase activity [396].

The present study has taken a different approach in the assessment of intestinal mast cell function in IBD by comparing enzymically dispersed colonic mucosal mast cells from patients with active Crohn's disease and ulcerative colitis with those from control subjects. Using an identical isolation procedure, the dispersion efficiency (histamine recovery) was significantly greater for inflamed colonic mucosa of patients from the two IBD groups ($P < 0.001$ in both cases); this observation may possibly be due to damage to the mucosal matrix normally linked to these inflammatory conditions. Moreover, it was estimated that a significantly greater number of mast cells were present per gram tissue in the colonic mucosa of both Crohn's and colitic patients as compared to control subjects. The increase in each case, however, was not accompanied by a corresponding rise in histamine content, a phenomenon that may be attributed to the significantly lower histamine content of the individual mast cells.

It has been reported by various authors [390,397], notably for Crohn's disease, that degranulated mast cells can be seen with some frequency in inflamed tissues under electron microscopy. The presence of this abundance of degranulated mast cells would indicate that they may play a role in the inflammatory process and, indeed, some of the histological features characteristic of IBD are most readily explained on this basis. Thus, the marked oedema could well be due to release of histamine from mast cells, while focal mucosal ulceration could result from the release of proteolytic enzymes. The present study, however, has shown that enzymically isolated colonic mucosal mast cells from patients with Crohn's disease and ulcerative colitis, along with those from control subjects, were structurally intact as judged under light microscopy and exhibited low spontaneous releases of histamine, thereby arguing against the theory that intestinal mast cells from inflamed tissues are particularly vulnerable and easily degranulated. However, it is also possible that degranulation of mast cells does occur but that these cells are lost during the isolation procedure.

The results in chapter six have clearly demonstrated that the predominant mast cell in the human colonic mucosa is one which is sensitive to formaldehyde fixation and stains positively with alcian blue but negatively with safranin. In the present study, the majority of colonic mucosal mast cells derived from inflamed tissues of patients with Crohn's disease and ulcerative colitis also shared these histochemical

characteristics. However, in contrast to control subjects, a significantly greater percentage of the formaldehyde insensitive, alcian blue positive subtype was observed in the two IBD groups. This may indicate the possible recruitment of a secondary group of mast cells, probably from the submucosa or, alternatively, a change in the local tissue microenvironment which has been demonstrated to be a crucial factor in the differentiation of mast cells into their respective phenotypes [266,289-292].

In a recent study, Fox et al [393] have shown that, upon immunological activation, enzymically dispersed intestinal mast cells from inflamed tissues of both Crohn's and colitic patients released a greater quantity of histamine, PGD_2 and LTC_4 when compared to those derived from normal tissues of the same subjects. With this in mind, they proposed that this may indicate an increased state of mast cell activation in areas of the intestine affected by the two diseases. The present study, however, has failed to confirm the results of Fox et al [393]. Histamine release induced by both anti-IgE and concanavalin A from dispersed colonic mucosal mast cells from patients with Crohn's disease and ulcerative colitis was comparable to that observed for controls. Moreover, these results are in accordance with the findings of Hőrauf et al [398] who, by using colonic mucosal biopsies, also failed to detect any difference in histamine secretion between controls and IBD patients upon stimulation with anti-IgE.

Isolated colonic mucosal mast cells from both Crohn's and colitic patients, similarly to those from control subjects, released comparable amount of histamine in response to challenge with the calcium ionophores A23187 and ionomycin. These results suggest that, in each case, an elevated level of the cation in the cell cytosol is a necessary and sufficient trigger for secretion [19,136,149]. Finally, the polybasic agent compound 48/80 and the neuropeptide substance P, potent releasers of histamine against rat serosal mast cells [chapters 3 and 4], were essentially ineffective against the three human colonic mast cell populations studied.

The management of IBD has historically been achieved by the use of steroids and sulphasalazine, a sulphonamide-related compound. However, although found to be effective in many subjects, these drugs fail to induce satisfactory remission in some patients even when given in high doses and over a long period of time. In addition,

unacceptable side effects often develop in association with prolonged usage of these compounds. These problems have therefore prompted the search for a drug which is more uniformly effective and free of unnecessary side effects. With this in mind, the prototype anti-allergic agent DSCG has been investigated extensively to see whether it may be of value in the management of these intestinal diseases.

Since its initial introduction into clinical practice more than 20 years ago, DSCG has been employed in the treatment of a number of allergic and inflammatory disorders ranging from asthma to food allergy [379]. Its clinical utility, however, in the management of IBD, has been the subject of many contrasting reports. Heatley and his colleagues [389] used DSCG for the treatment of 26 patients with chronic ulcerative colitis and reported considerable clinical and sigmoidoscopic improvement in 14 subjects. Mani et al [399], in a similar study, also observed significant positive changes in many patients' sense of well being together with improvement in their sigmoidoscopic appearance. More recently, Grace et al [387], in an enema study comparing the potency of DSCG and prednisolone found the chromone to be as effective as the steroidal agent in the management of patients with ulcerative colitis. In keeping with the same theme, Sidorov and Marcon [400] found that in a group of patients that did not respond to either steroids or sulphasalazine, 41 % of them experienced definite symptomatic and sigmoidoscopic remission when given DSCG. However, in contrast to these beneficial reports of DSCG in the treatment of ulcerative colitis, both Bucknell et al [401] and Binder et al [402] found that there was no evidence of an improved clinical response in patients with the active disease when compared with placebo. In addition, Dronfield et al [403] and Willoughby et al [404] both reported that DSCG was far less effective than sulphasalazine in the maintenance of remission in colitic patients. There have been far fewer studies in the literature concerning the use of DSCG in the treatment of Crohn's disease and these have shown the chromone to be no better than placebo [402,405].

The many contrasting reports in the literature have therefore questioned the effectiveness of DSCG in the management of IBD. However, it was pointed out by Grace et al [387] that in those studies where DSCG was found to be inactive, the chromone was administered orally to patients rather than by the enema method. In

view of its known topical action on the bronchial mucosa, it is not unreasonable to suggest that DSCG will also exert its effect by topical action on the intestinal mucosa. The concentration required in the lumen of the intestine to produce a therapeutic effect in IBD is not known but it is possible that this is not reached when DSCG is taken orally. To reinforce their theory, Grace et al [387] showed that when given in an enema form, DSCG was as effective as the steroid prednisolone in the treatment of patients with ulcerative colitis.

Although the expression of IBD is likely to be multifactorial, the potential role played by the intestinal mast cell in these conditions should not be discarded especially in view of its unique location, apparent increase in number as demonstrated in this study and its ability to release a variety of chemotactic and spasmogenic mediators. With this in mind, agents that have the ability to inhibit mediator secretion from these cells may be of therapeutic value in the management of IBD.

The present study has shown that both DSCG and nedocromil sodium were moderatively effective in inhibiting anti-IgE-induced histamine release from enzymically dispersed colonic mucosal mast cells from patients with Crohn's disease and ulcerative colitis. The average maximum inhibition achieved, in each case, was greater or comparable to that observed for control subjects. However, the inhibitory effect produced by the two chromones against mast cells from Crohn's patients was significantly reduced upon an initial preincubation, thus indicating, as discussed previously, the possible recruitment of a secondary group of mast cells, probably from the submucosa. In addition, this tachyphylactic phenomenon may also explain the apparent lack of response of Crohn's patients towards DSCG in studies in vivo [402,405]. In contrast, the inhibition observed against mast cells from colitic patients was not affected with preincubation. This suggests that, if the chromones can be administered in an appropriate form (e.g. by enema) so that an adequate concentration can be reached in the intestinal mucosa, both DSCG and nedocromil sodium may well have some clinical value in the treatment of this disorder. Indeed, the same argument can also be applied to LU 48953 and to a lesser extent MY 1250. Moreover, the two compounds have the additional advantage that they do not exhibit tachyphylaxis against colonic mast cells from Crohn's patients.

Finally, similarly to those from control subjects, the release process from colonic mucosal mast cells of patients with Crohn's disease and ulcerative colitis was subject to modulation by cAMP-active compounds. In each case, the phosphodiesterase inhibitor theophylline was more potent than the β-adrenoceptor agonists isoprenaline and salbutamol. This observation gives further support to the hypothesis that a large fraction of the immunologically released histamine in human mast cells is insulated from β-adrenergic modulation.

In summary, using an identical dispersion procedure, the present study has demonstrated an increase in mast cell numbers in the inflamed colonic mucosa of patients with active Crohn's disease and ulcerative colitis as compared to control subjects. In addition, a significantly greater percentage of the formaldehyde insensitive, alcian blue positive subtype was observed in both IBD groups. This may therefore suggest the recruitment of a secondary group of mast cells, a phenomenon analogous to that described by Enerbäck et al [406,407] for the redistribution of nasal mast cells in seasonal allergic rhinitis. Despite this, colonic mucosal mast cells from both Crohn's and colitic patients were, in general, functionally similar to those derived from control subjects in their response to a number of immunological and non-immunological secretagogues as well as to agents that suppressed mediator release. In total, although the present study failed to demonstrate an increase in reactivity of the colonic mucosal mast cell in IBD, this finding should not detract from the potential role it may have in these disorders.

Fig 8.1 Histamine release induced by (a) anti-human IgE (n=4-9) and (b) concanavalin A (n=4-7) from isolated human colonic mucosal mast cells from control patients (CR) and patients with Crohn's disease (CD) and ulcerative colitis (UC).

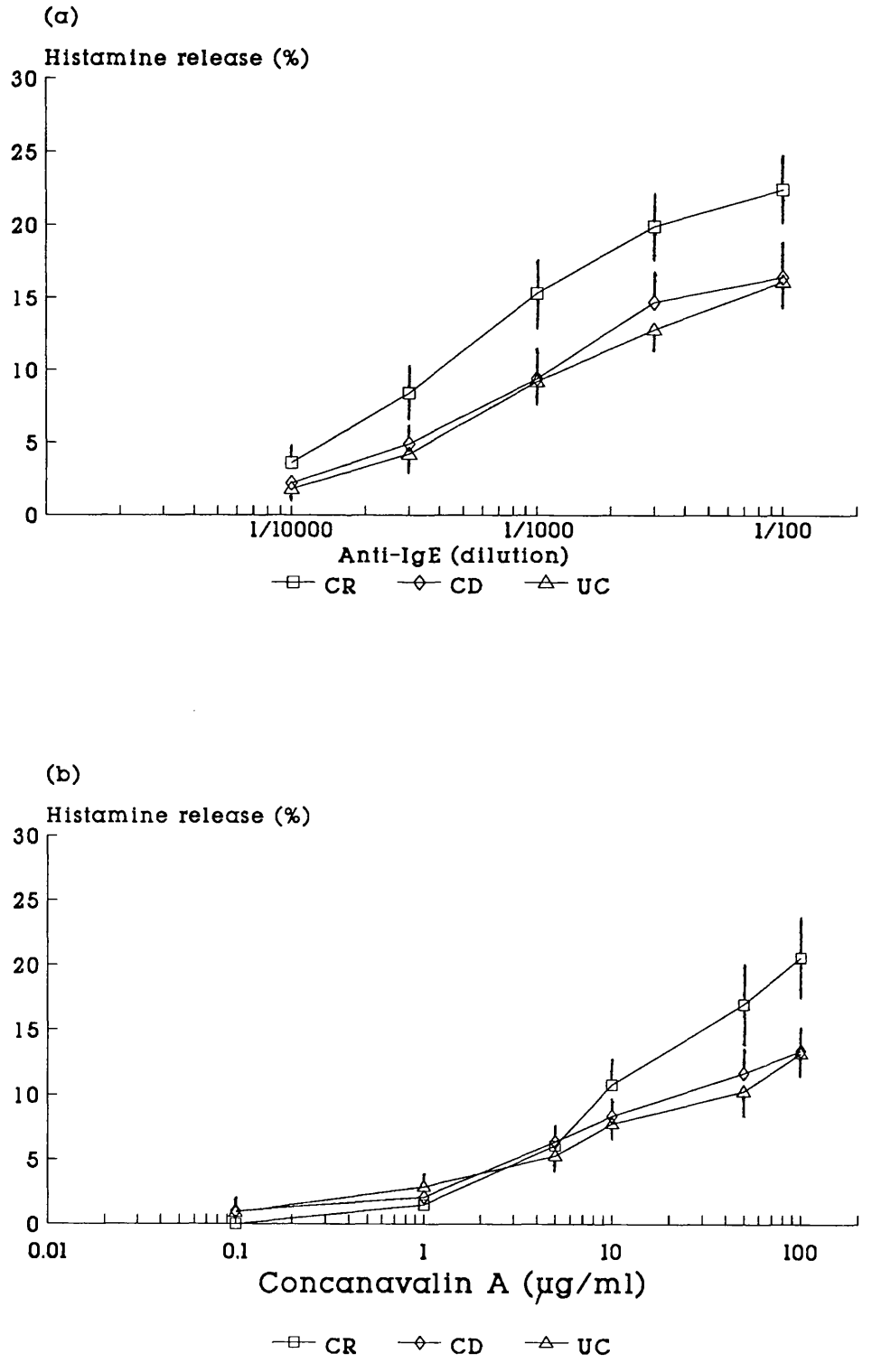
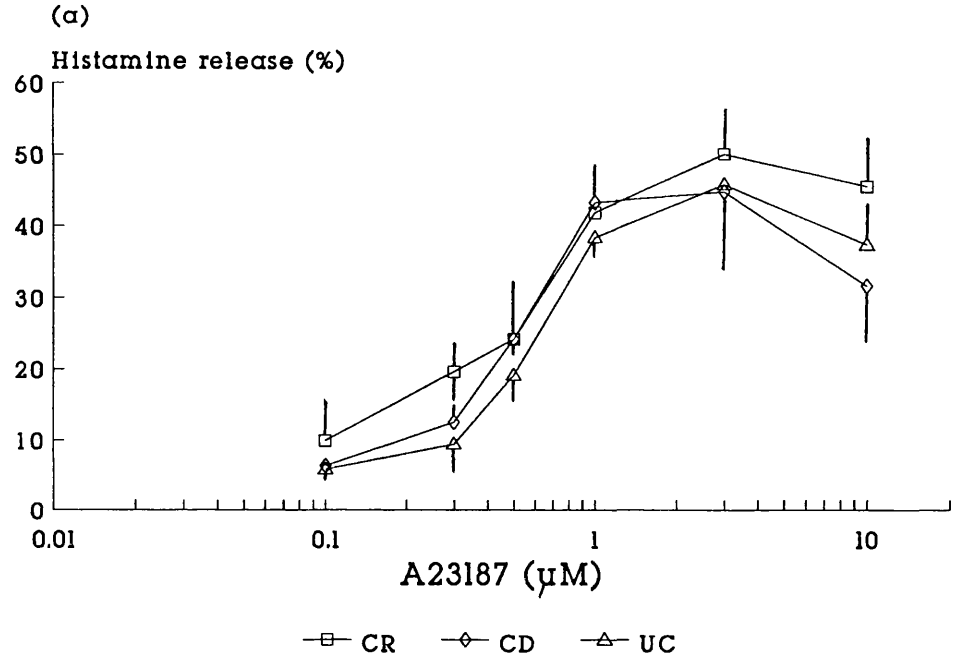


Fig 8.2 Histamine release induced by (a) A23187 (n=3-8) and (b) ionomycin (n=3-6) from isolated human colonic mucosal mast cells from control patients (CR) and patients with Crohn's disease (CD) and ulcerative colitis (UC).



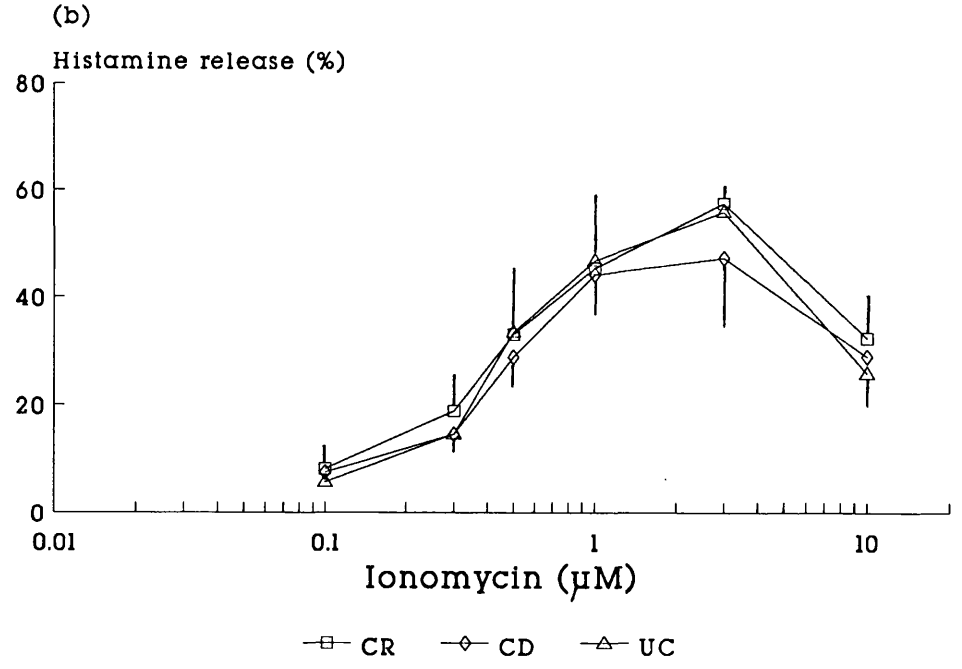


Fig 8.3 Effect of disodium cromoglycate (DSCG) on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from control patients (CR) and patients with Crohn's disease (CD). The drug was added simultaneously with stimulus to cells. The control releases were 14.7±3.1 %, n=6, CR and 16.7±2.6 %, n=5, CD.

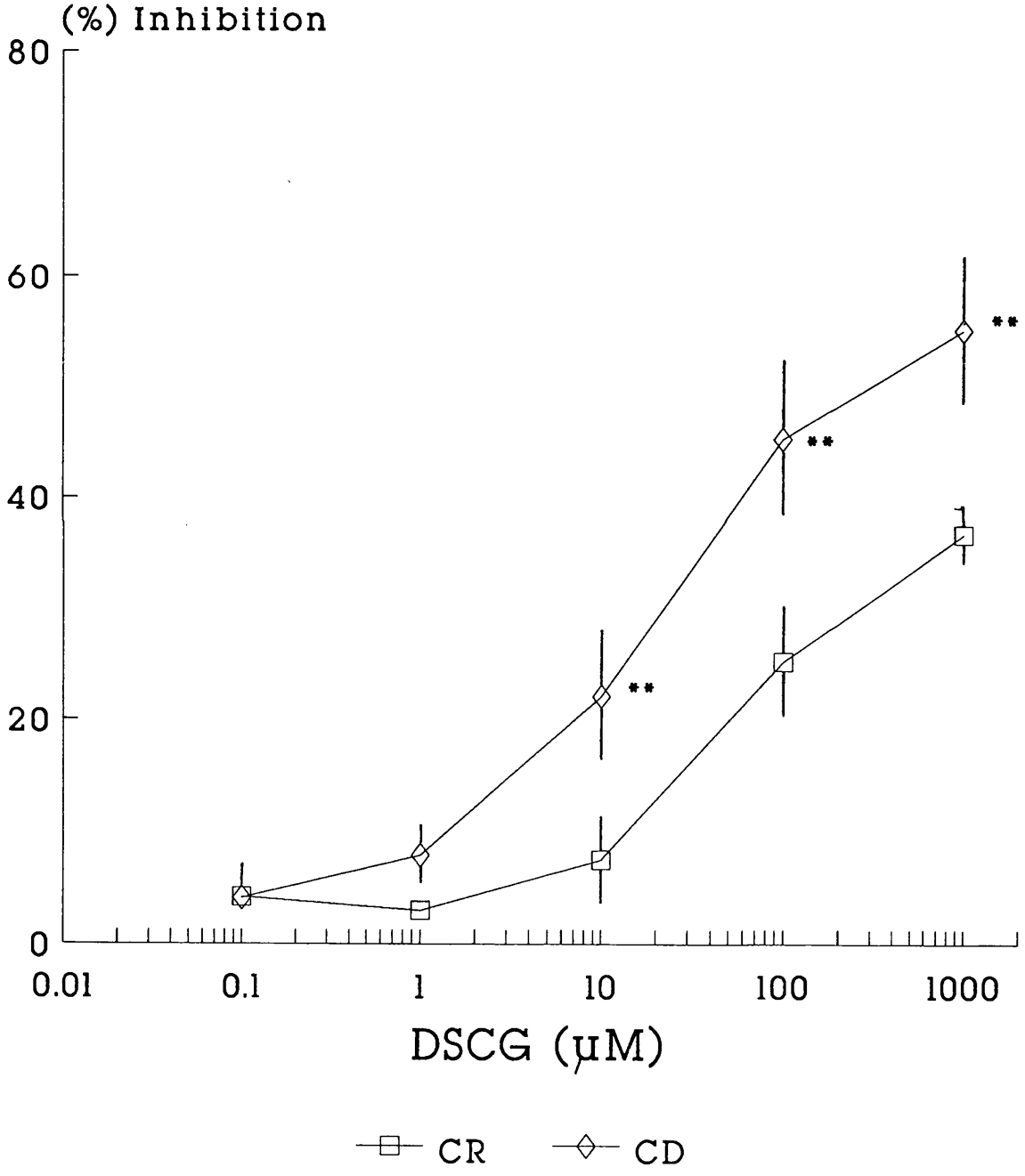


Fig 8.4 Effect of disodium cromoglycate (DSCG) on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from control patients (CR) and patients with ulcerative colitis (UC). The drug was added simultaneously with stimulus to cells. The control releases were 14.7±3.1 %, n=6, CR and 16.5±5.1 %, n=4, UC.

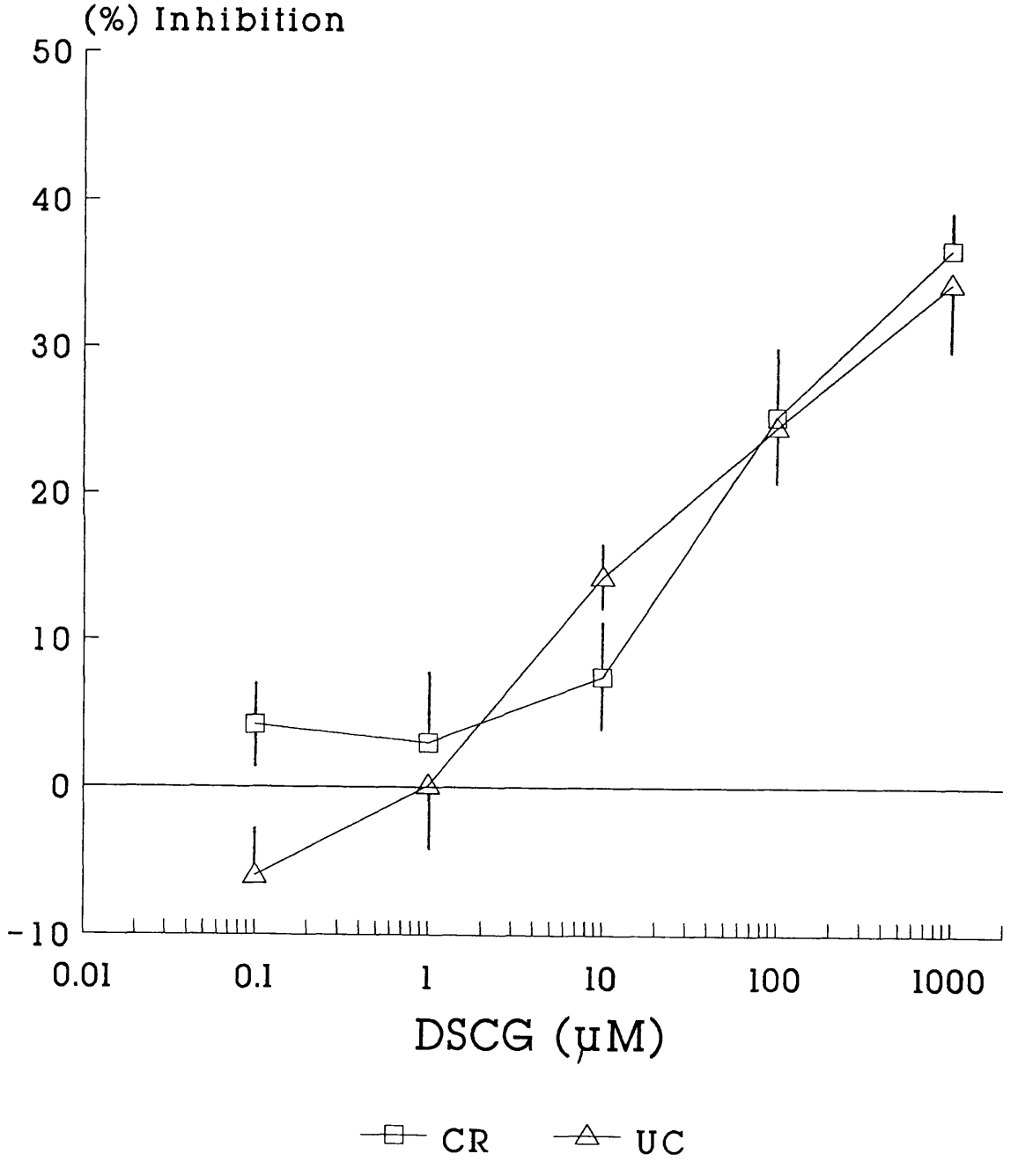


Fig 8.5 Effect of disodium cromoglycate (DSCG) on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from control patients. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 14.7±3.1 % (0') and 14.9±2.2 % (10') respectively, n=6, paired data.

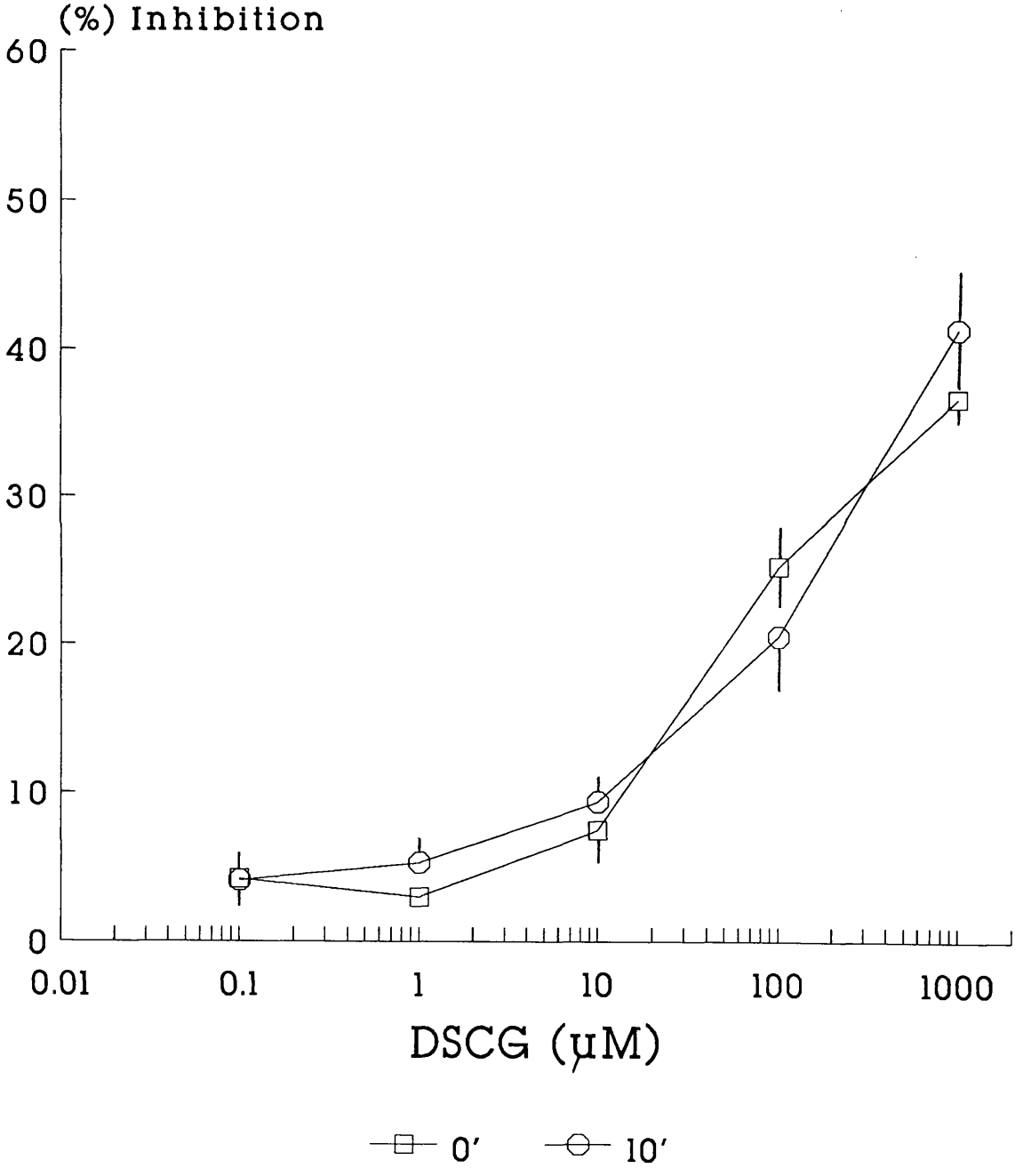


Fig 8.6 Effect of disodium cromoglycate (DSCG) on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from patients with Crohn's disease. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 16.7±2.6 % (0') and 16.8±2.5 % (10') respectively, n=5, paired data.

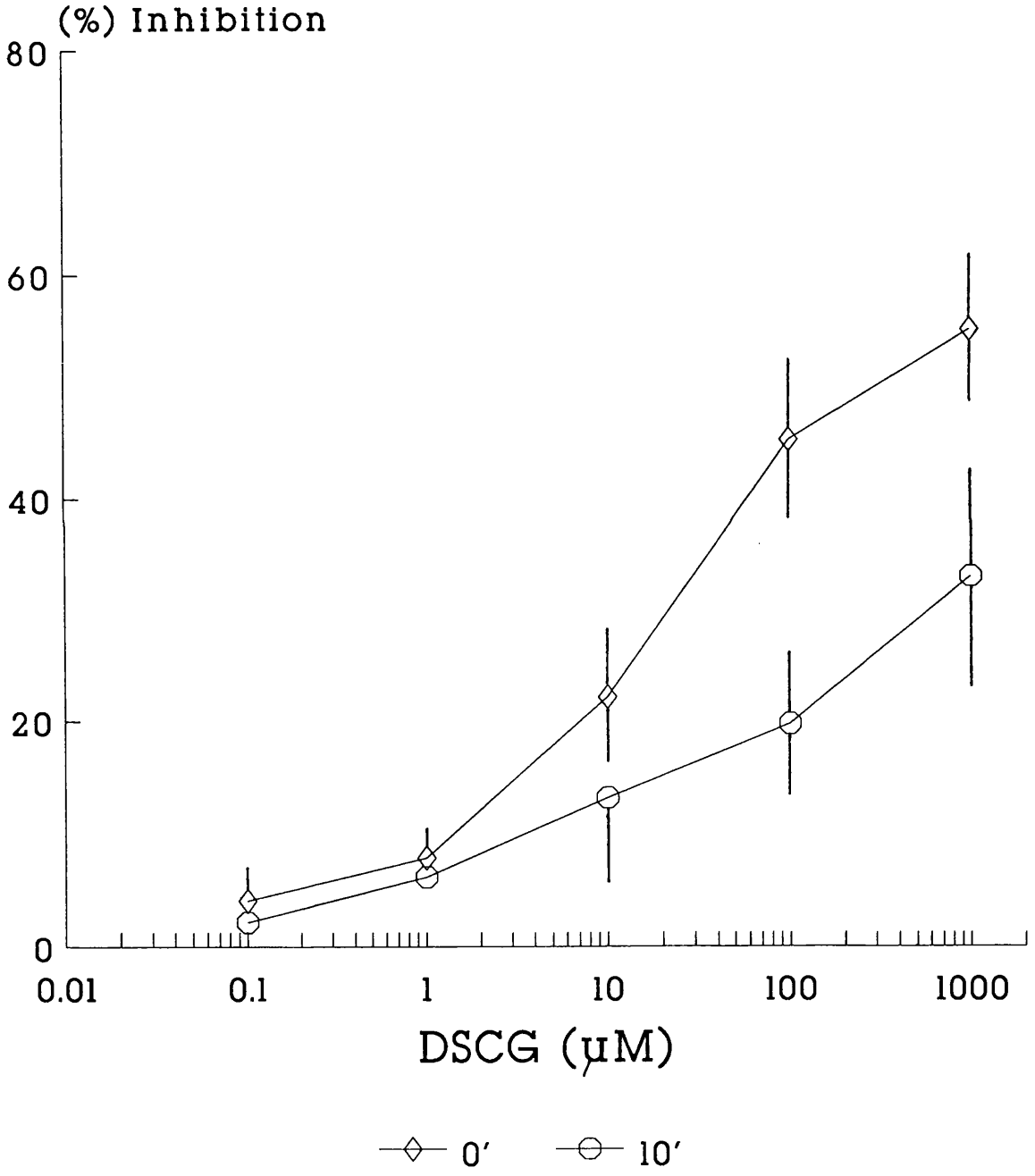


Fig 8.7 Effect of disodium cromoglycate (DSCG) on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from patients with ulcerative colitis. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 16.5±5.1 % (0') and 17.5±5.1 % (10') respectively, n=4, paired data.

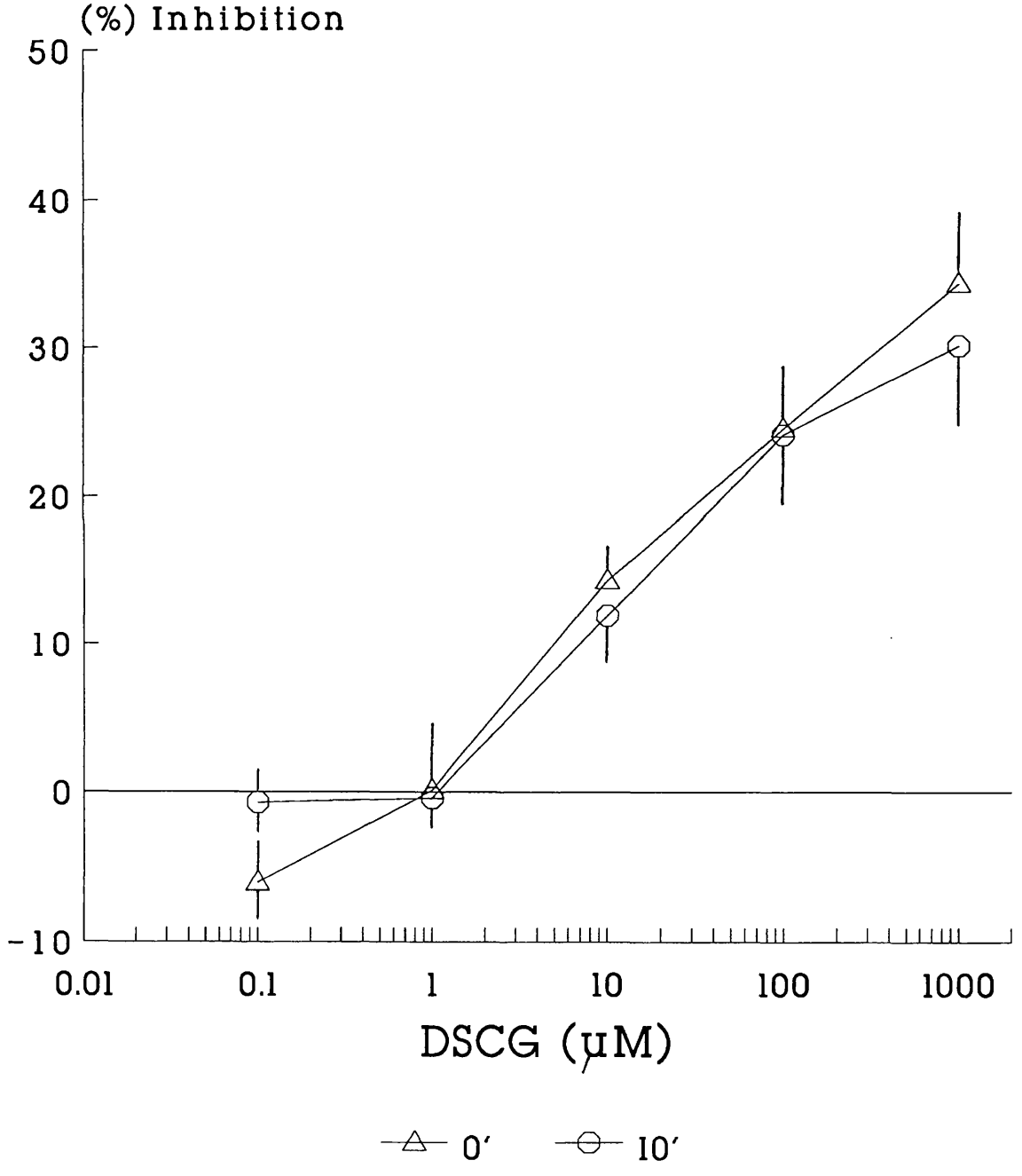


Fig 8.8 Effect of nedocromil sodium on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from control patients (CR) and patients with Crohn's disease (CD). The drug was added simultaneously with stimulus to cells. The control releases were 12.1±0.7 %, n=6, CR and 13.1±1.2 %, n=4, CD.

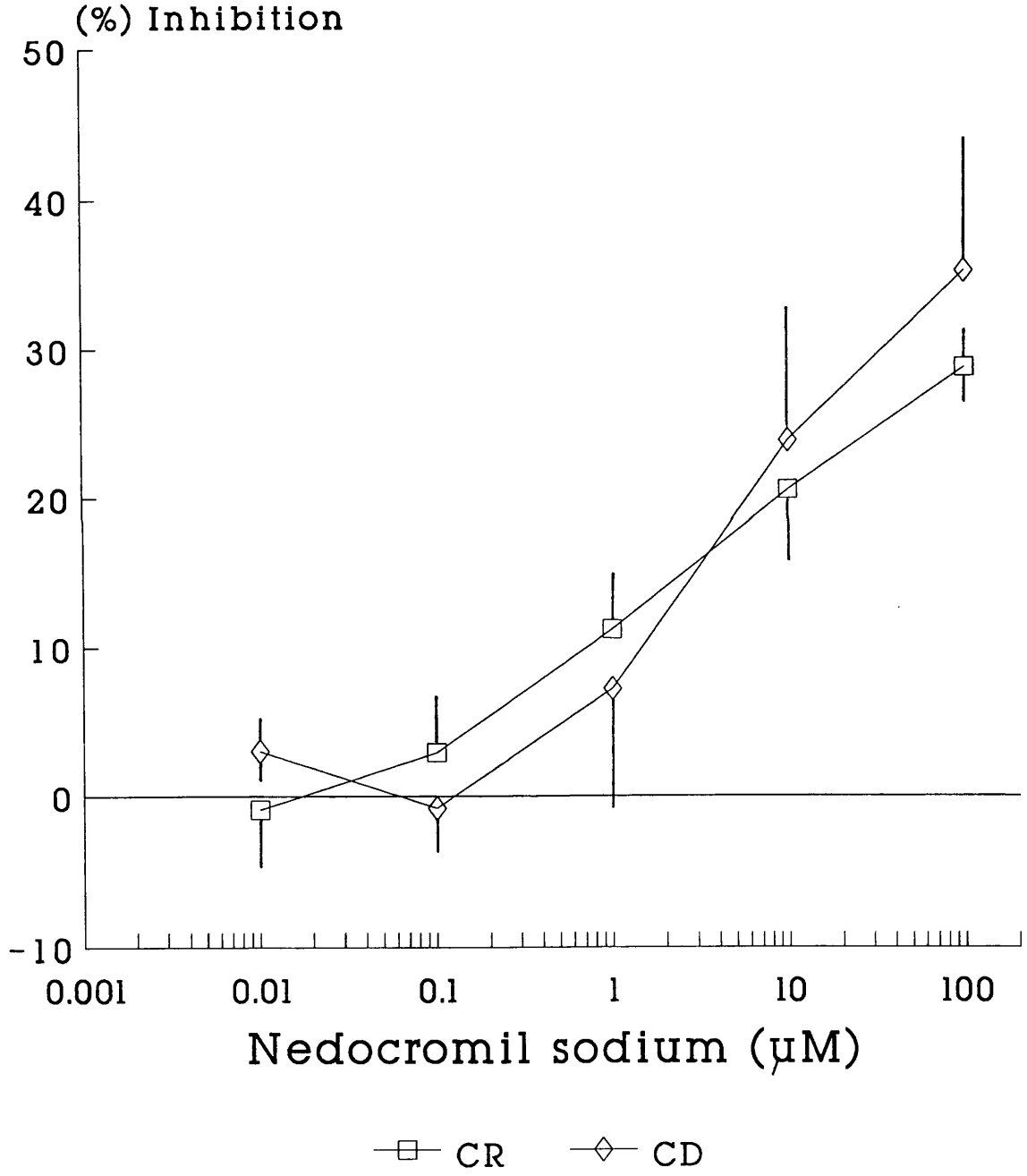


Fig 8.9 Effect of nedocromil sodium on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from control patients (CR) and patients with ulcerative colitis (UC). The drug was added simultaneously with stimulus to cells. The control releases were 12.1±0.7 %, n=6, CR and 19.9±8.5 %, n=4, UC.

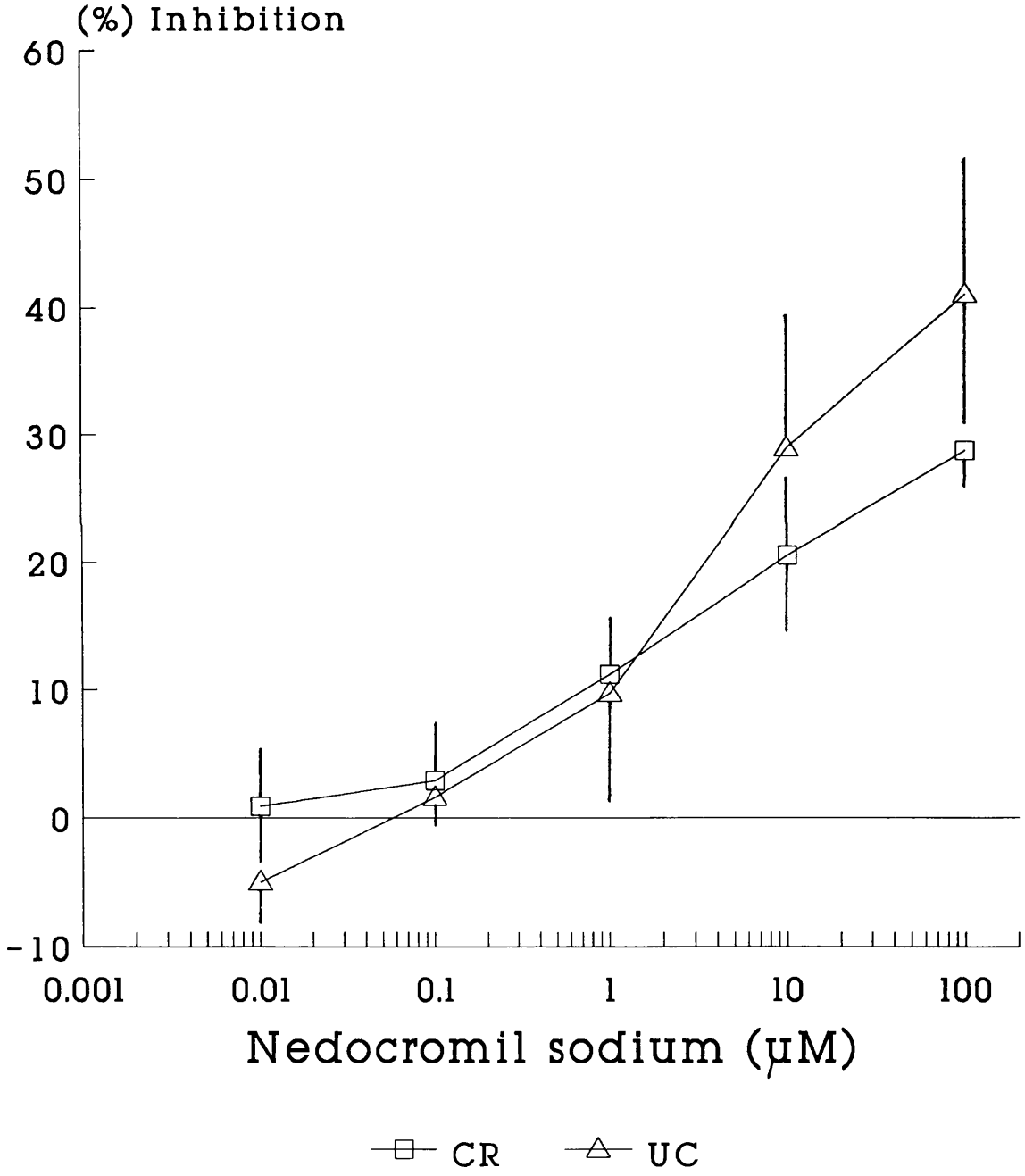


Fig 8.10 Effect of nedocromil sodium on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from control patients. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 12.1±0.7 % (0') and 13.1±1.2 % (10') respectively, n=6, paired data.

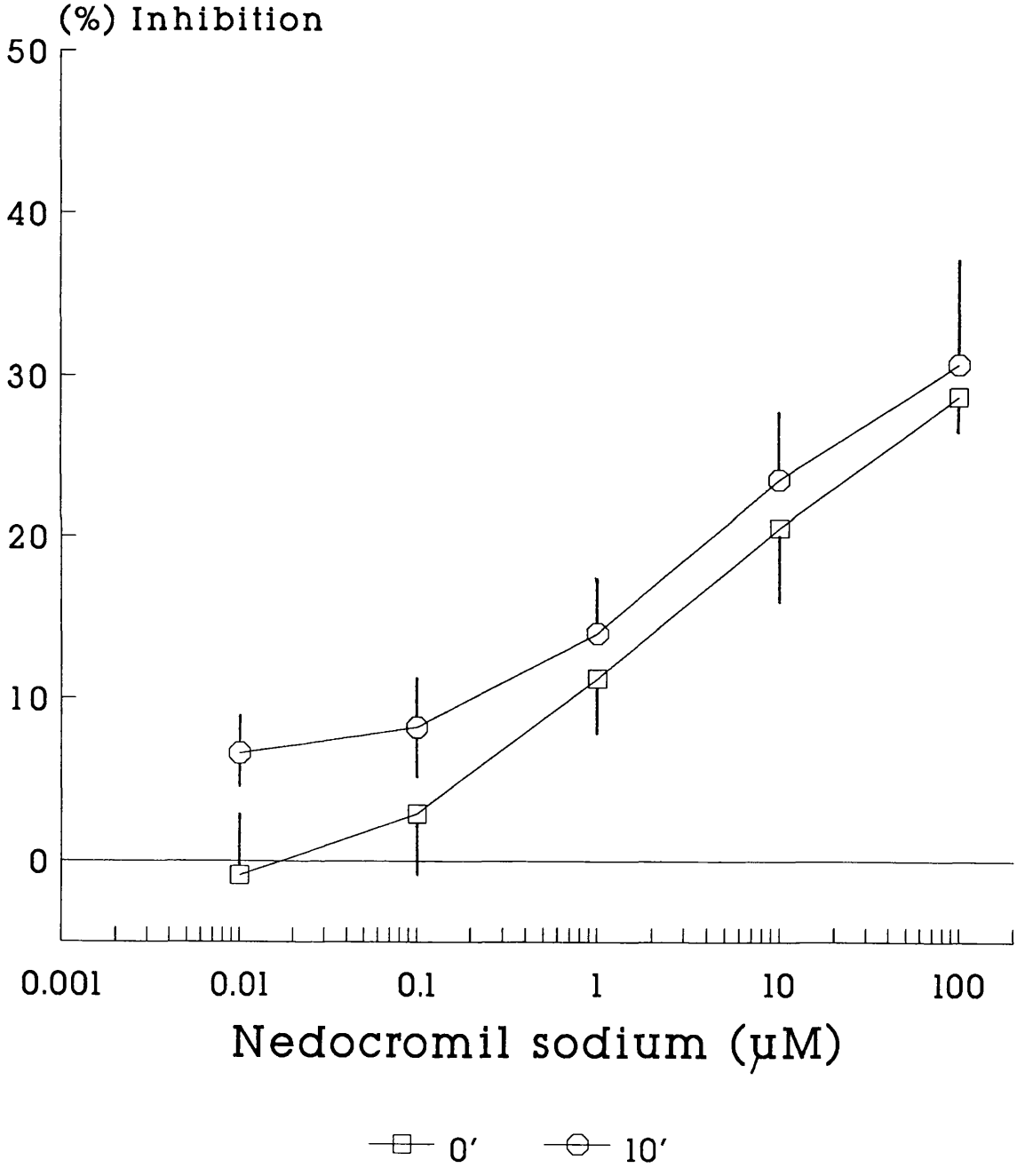


Fig 8.11 Effect of nedocromil sodium on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from patients with Crohn's disease. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 15.7±3.1 % (0') and 15.9±2.9 % (10') respectively, n=4, paired data.

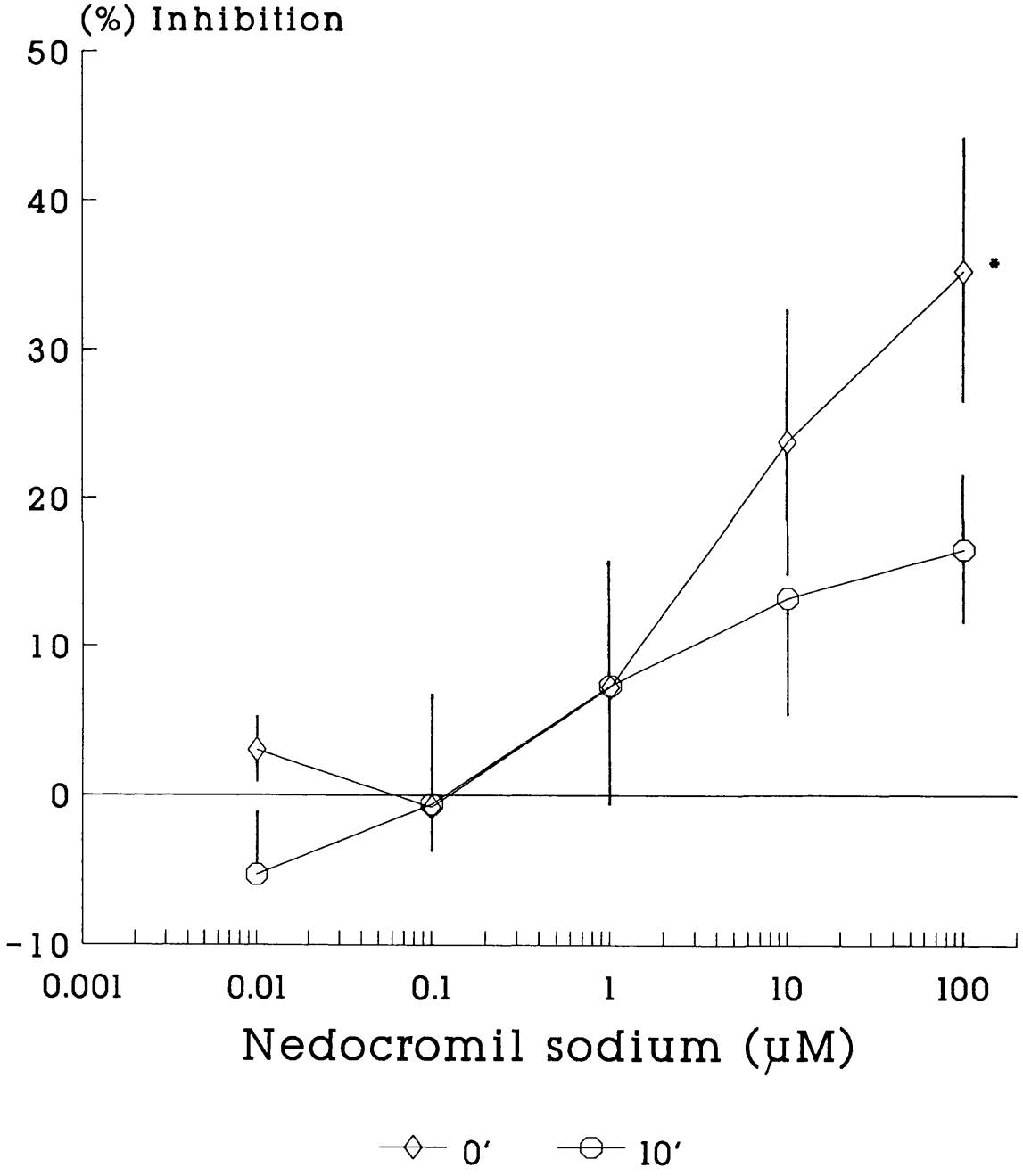


Fig 8.12 Effect of nedocromil sodium on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from patients with ulcerative colitis. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 19.9±8.5 % (0') and 20.7±8.3 % (10') respectively, n=4, paired data.

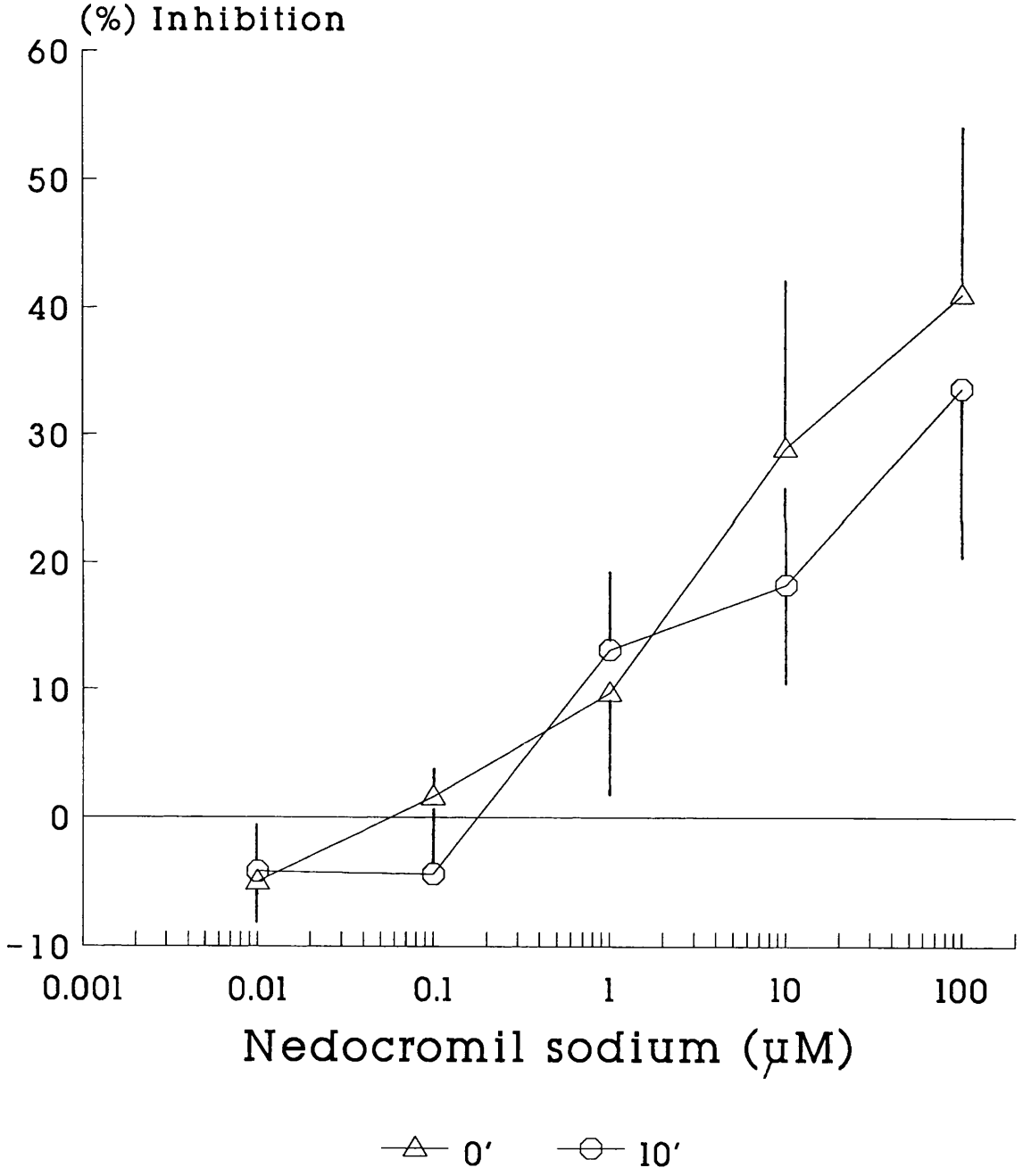


Fig 8.13 Effect of LU 48953 on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from control patients (CR) and patients with Crohn's disease (CD) and ulcerative colitis (UC). The drug was added simultaneously with stimulus to cells. The control releases were 14.7±3.1 %, n=6, CR; 16.7±2.6 %, n=5, CD and 16.5±5.1 %, n=4, UC.

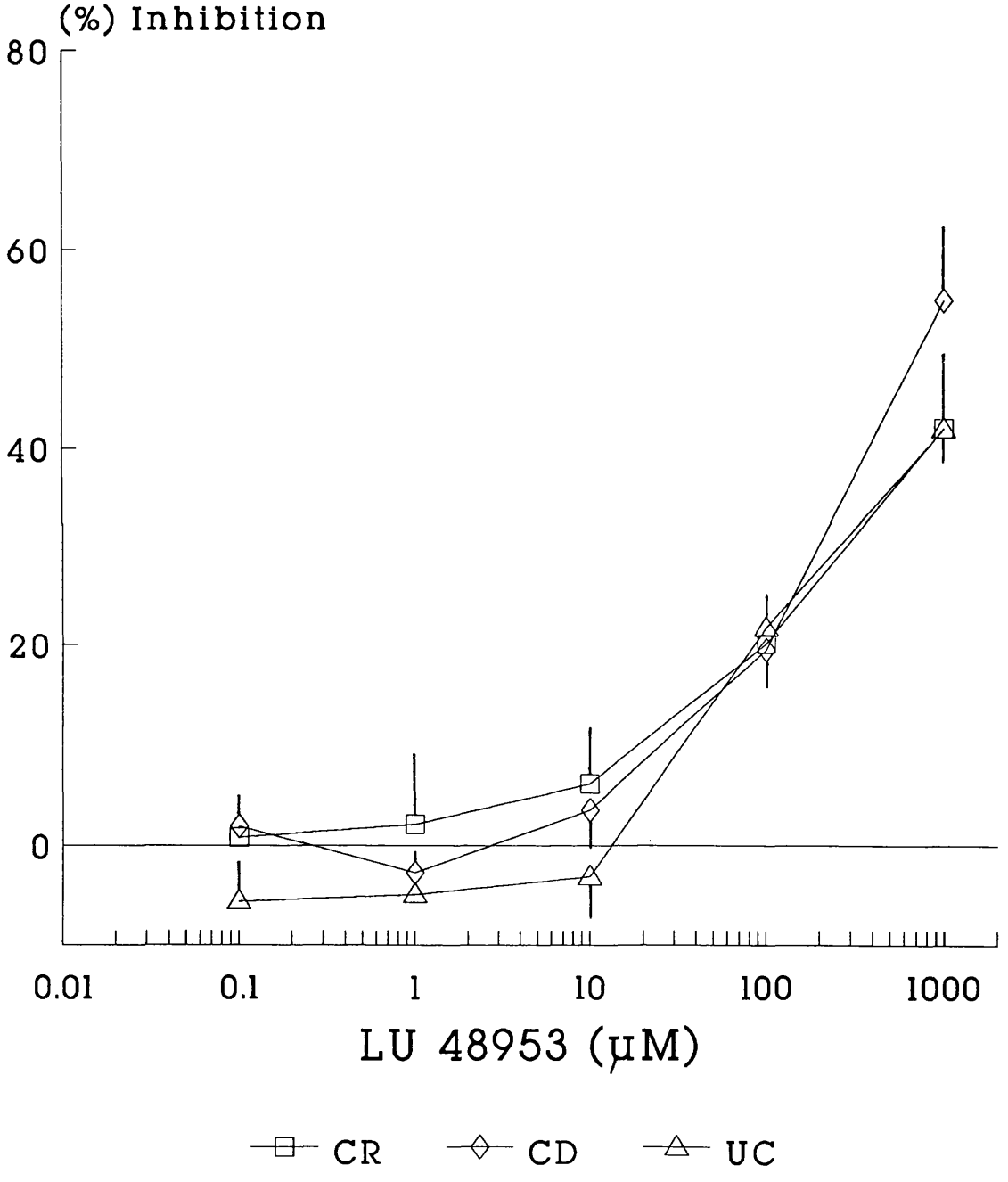


Fig 8.14 Effect of LU 48953 on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from control patients. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 14.7±3.1 % (0') and 14.9±2.2 % (10') respectively, n=6, paired data.

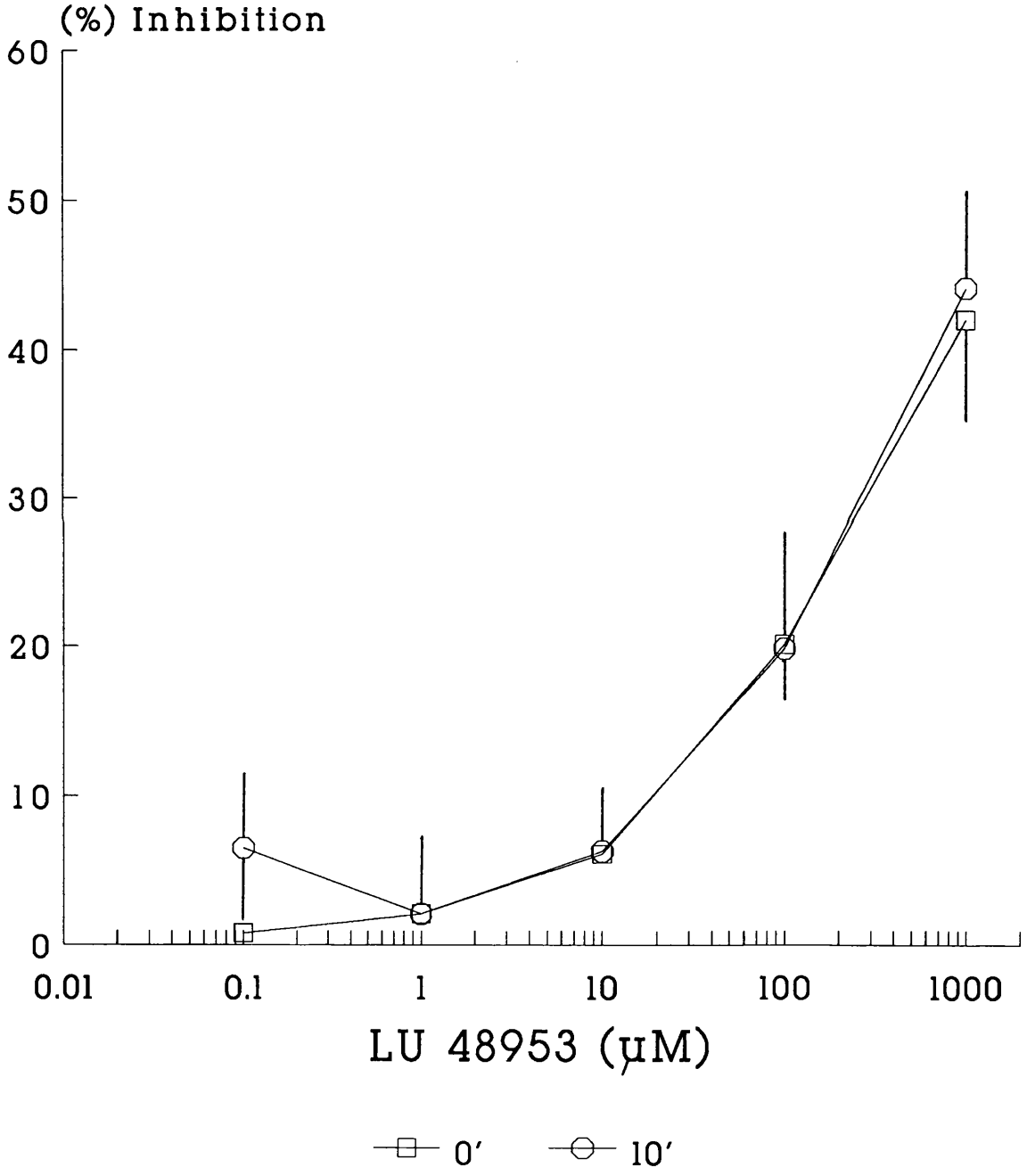


Fig 8.15 Effect of LU 48953 on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from patients with Crohn's disease. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 16.7±2.6 % (0') and 16.8±2.5 % (10') respectively, n=5, paired data.

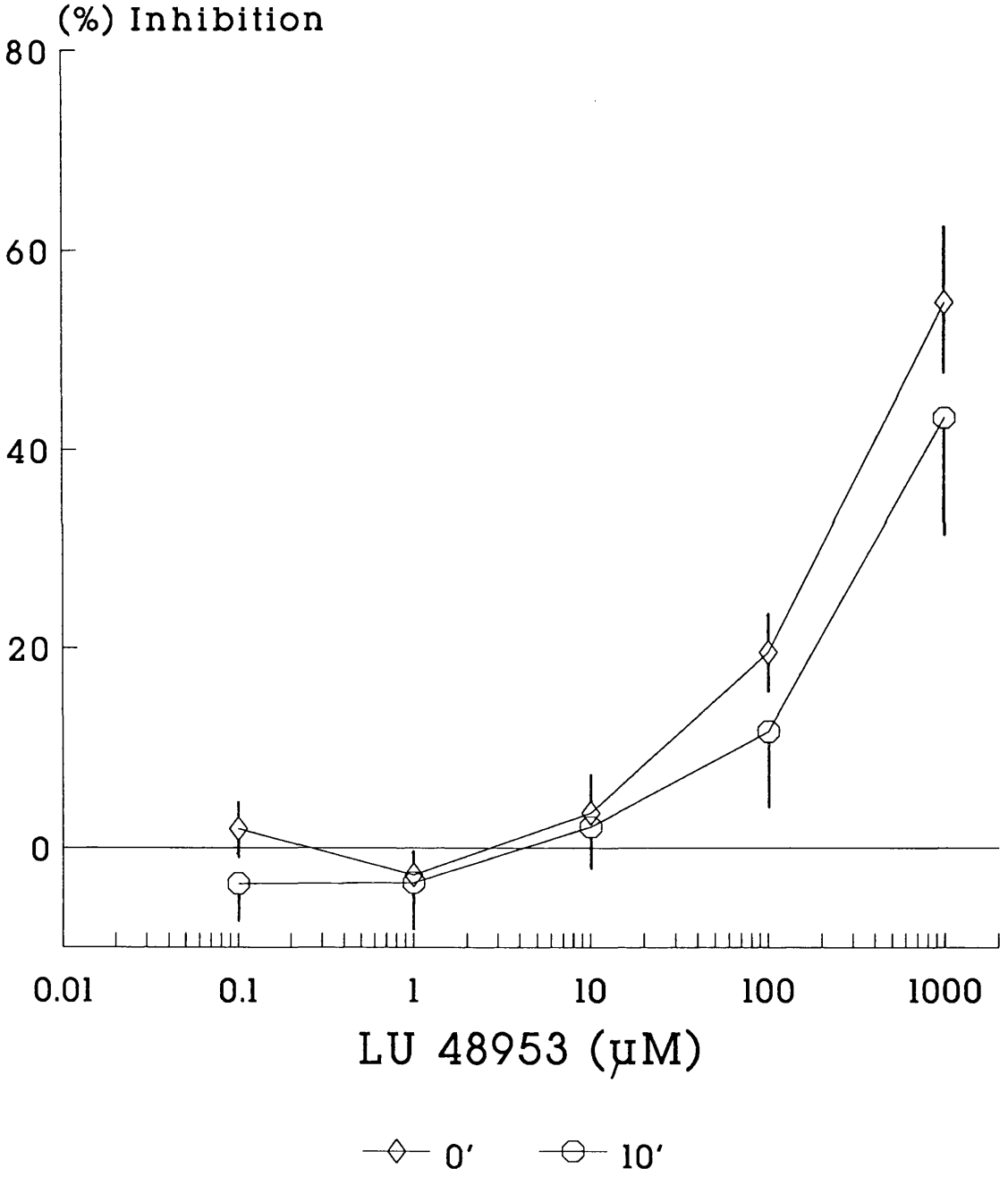


Fig 8.16 Effect of LU 48953 on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from patients with ulcerative colitis. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 16.5±5.1 % (0') and 17.5±5.1 % (10') respectively, n=4, paired data.

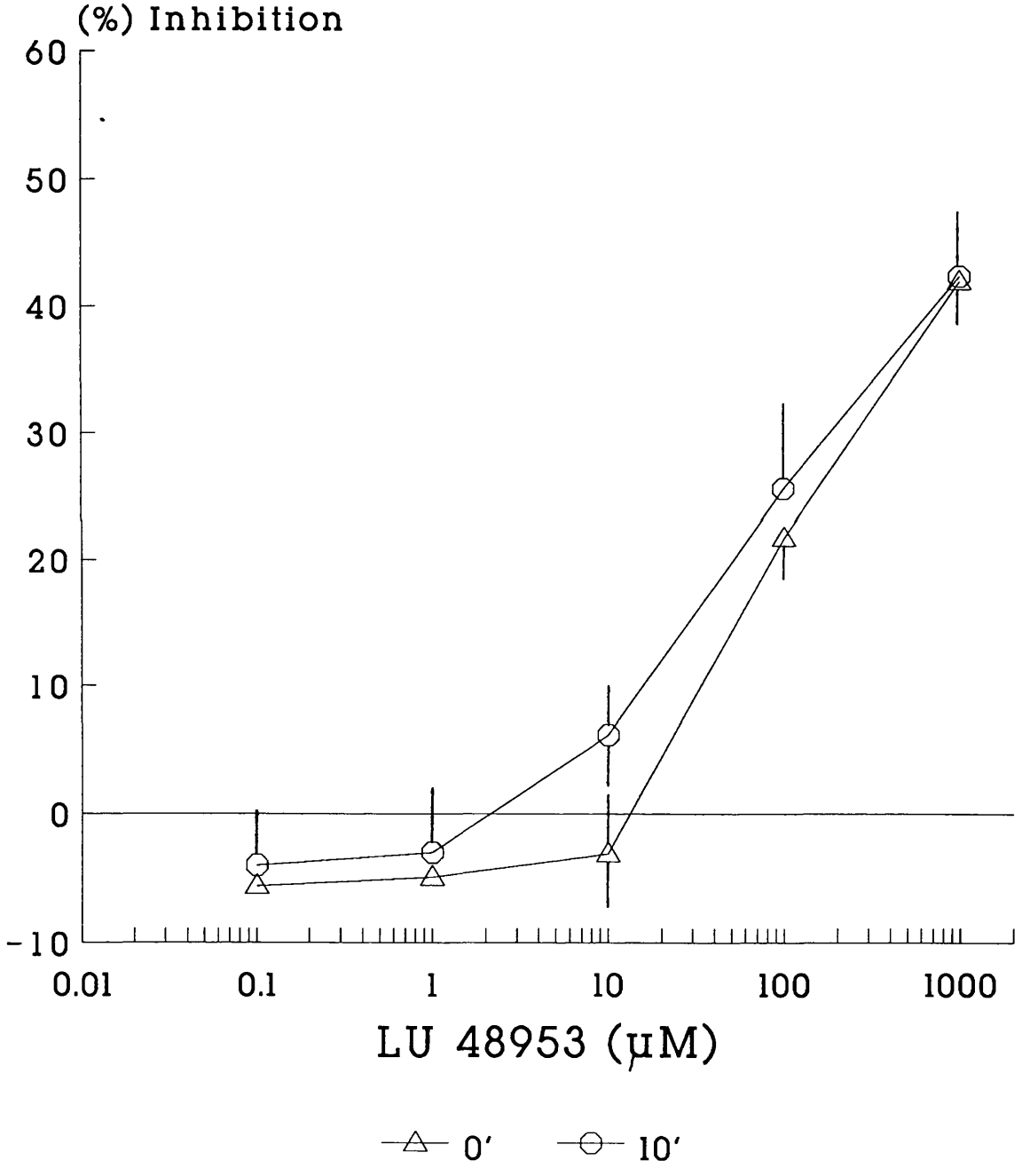


Fig 8.17 Effect of MY 1250 on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from control patients (CR) and patients with Crohn's disease (CD) and ulcerative colitis (UC). The drug was added simultaneously with stimulus to cells. The control releases were 15.7±2.8 %, n=6, CR; 15.7±3.1 %, n=4, CD and 22.3±7.0 %, n=5, UC.

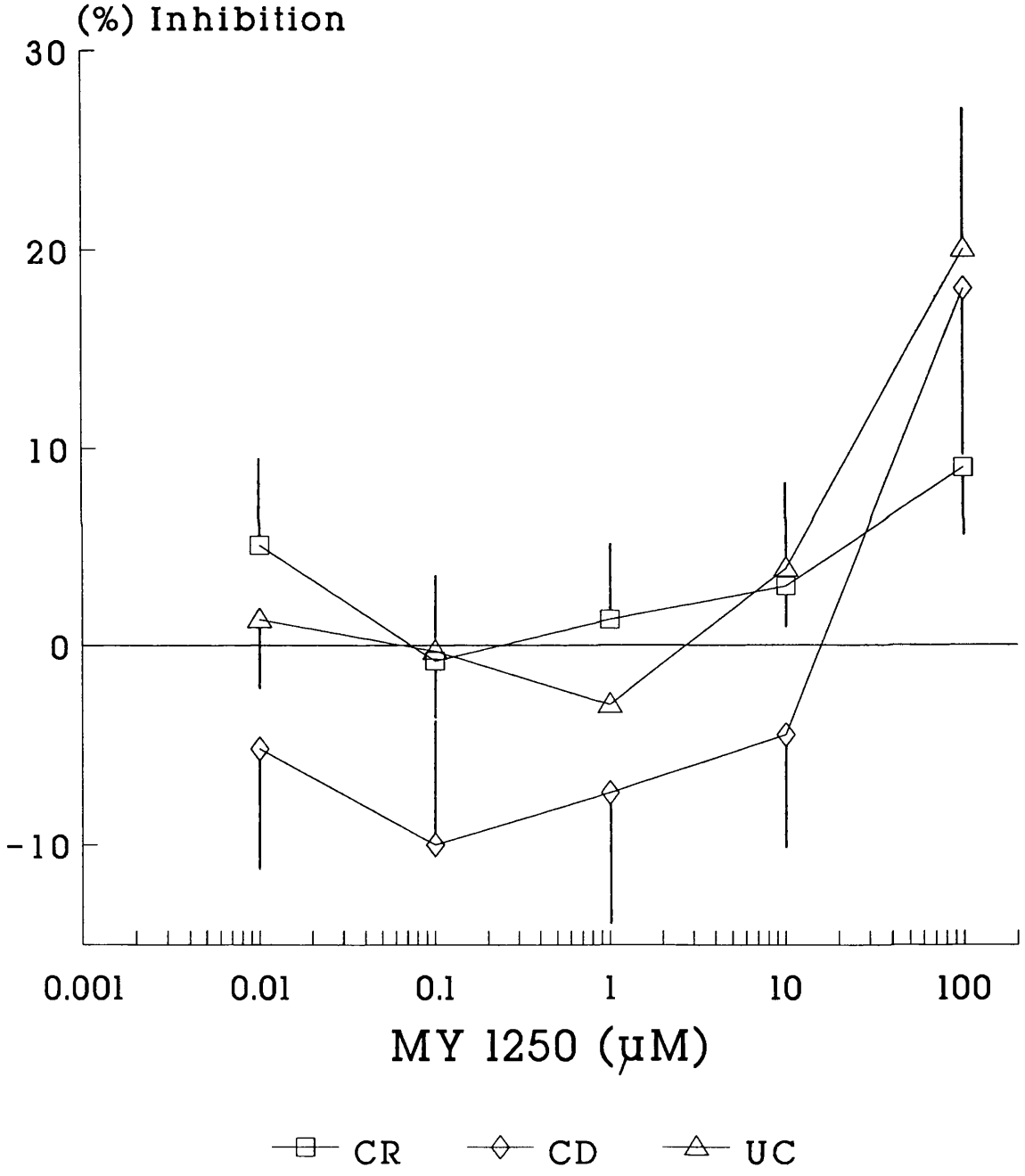


Fig 8.18 Effect of MY 1250 on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from control patients. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 15.7±2.8 % (0') and 15.8±1.9 % (10') respectively, n=6, paired data.

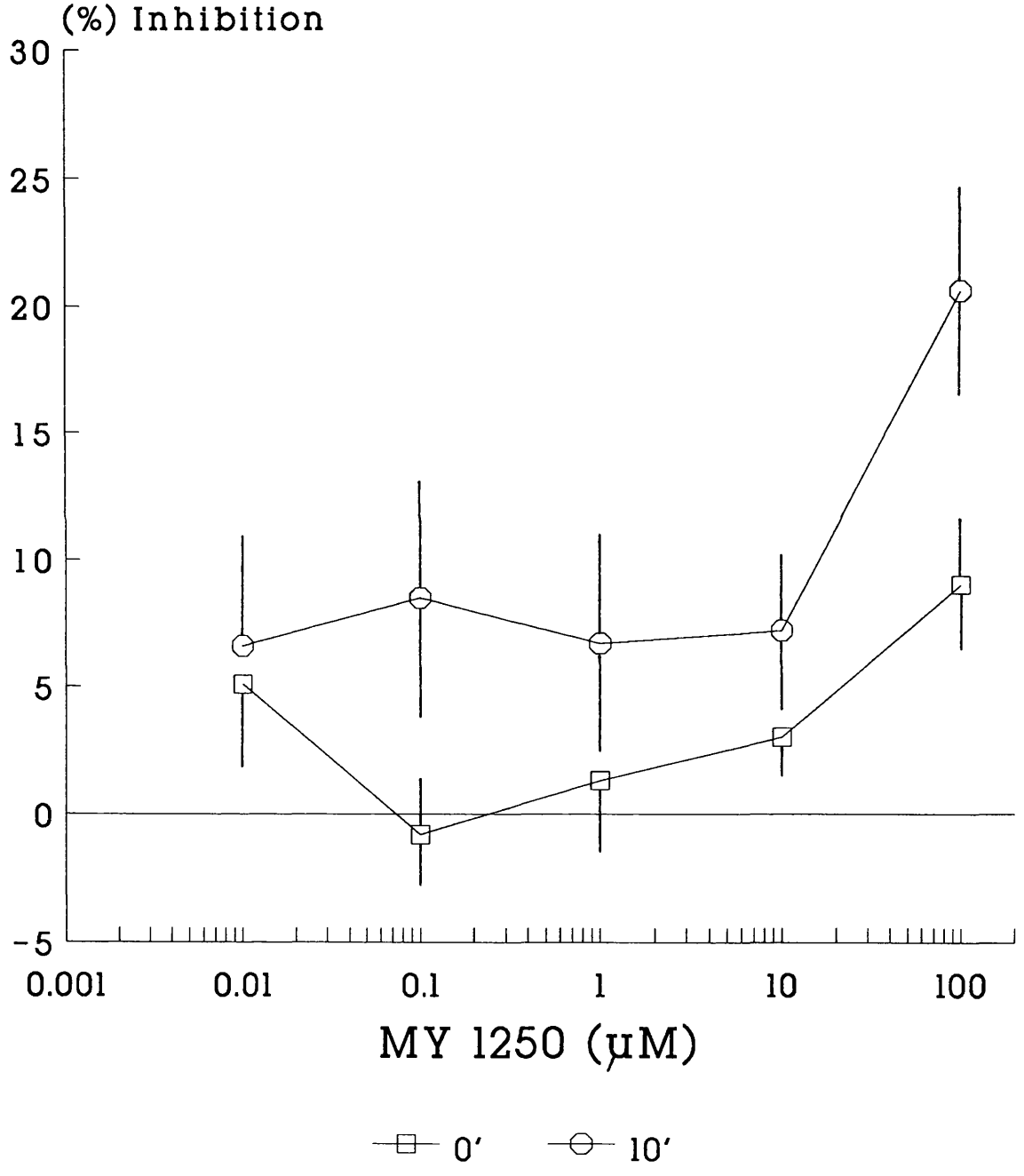


Fig 8.19 Effect of MY 1250 on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from patients with Crohn's disease. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 15.7±3.1 % (0') and 15.9±2.9 % (10') respectively, n=4, paired data.

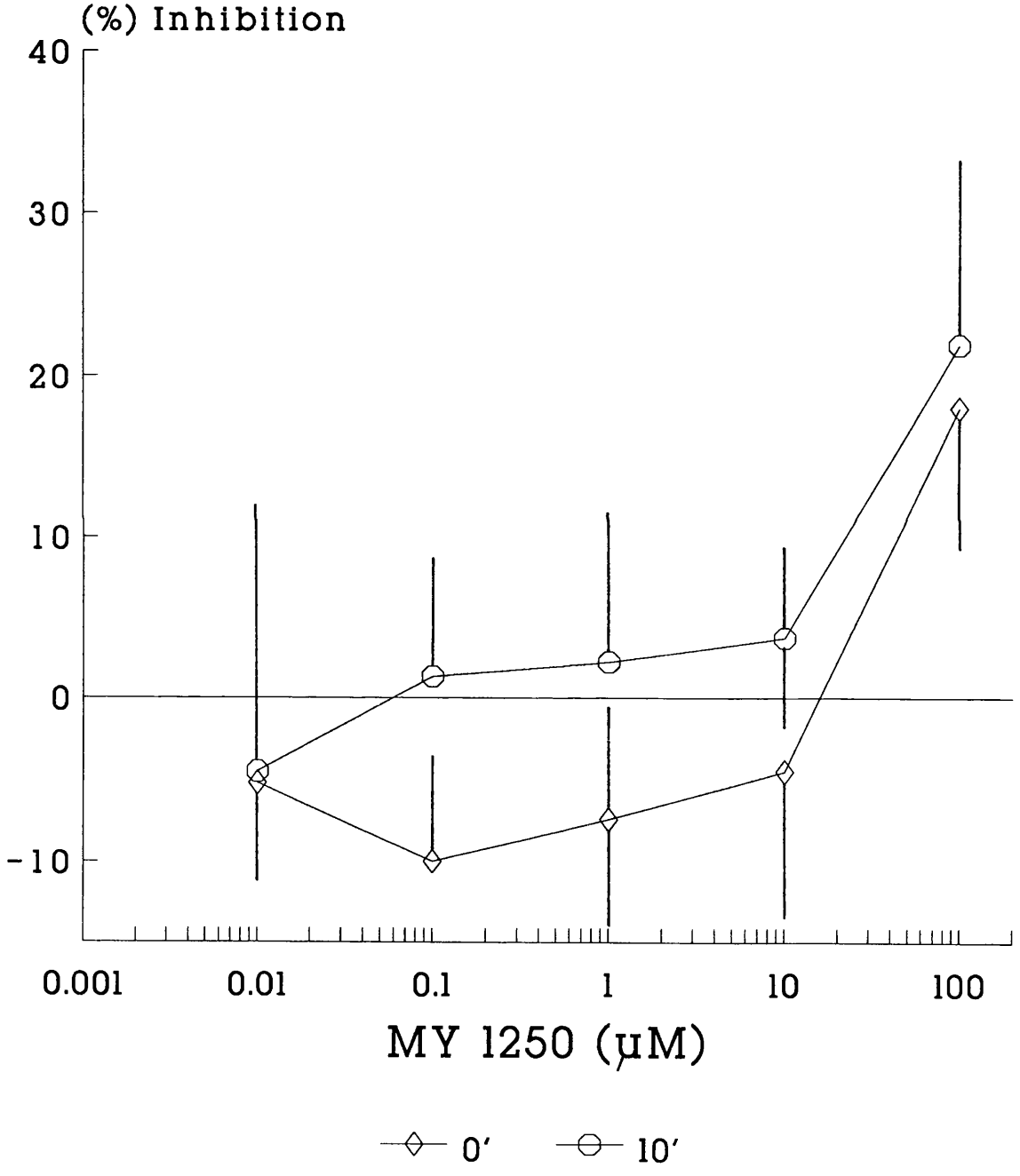


Fig 8.20 Effect of MY 1250 on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from patients with ulcerative colitis. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 22.3±7.0 % (0') and 23.1±6.9 % (10') respectively, n=5, paired data.

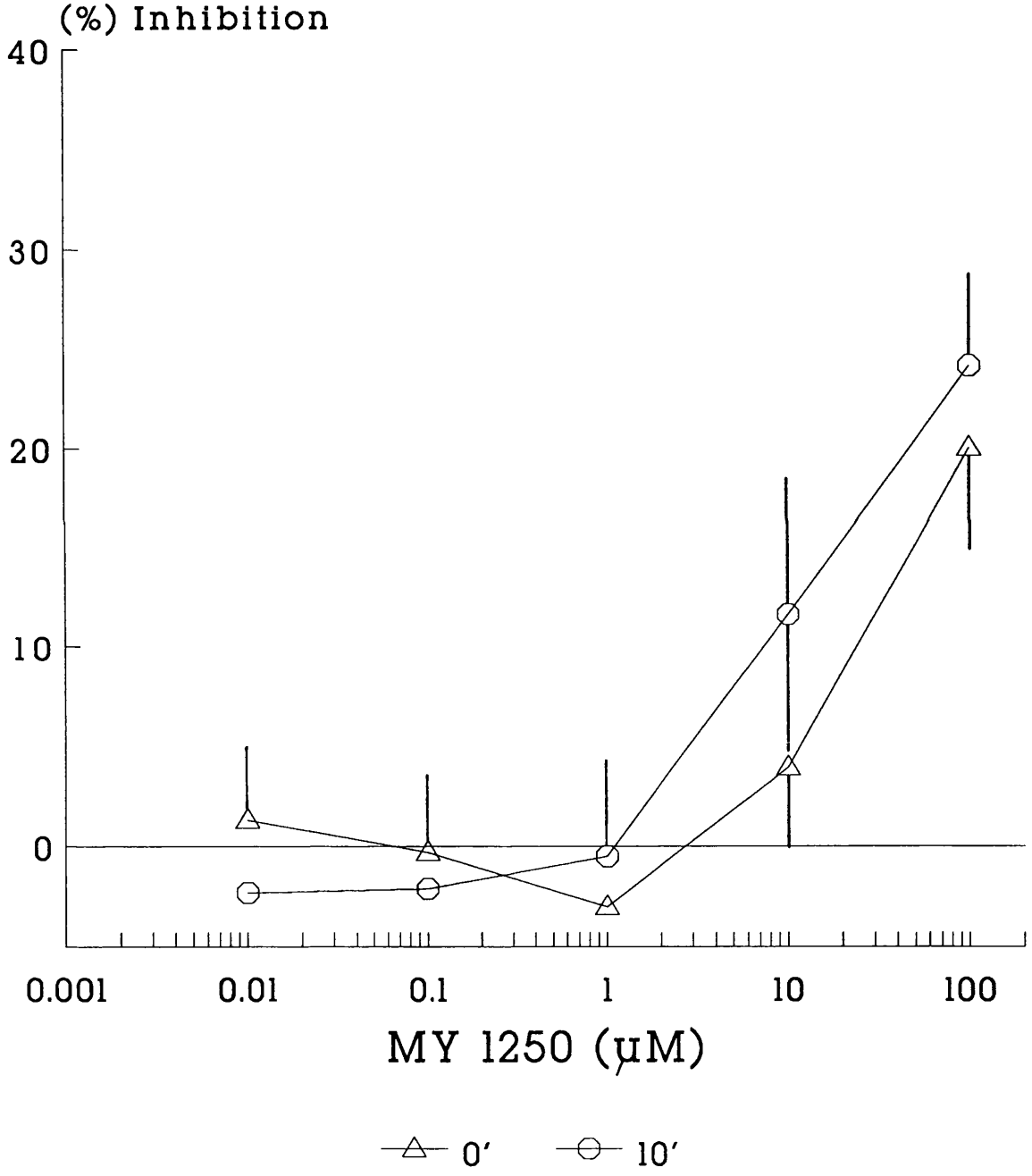


Fig 8.21 Effect of theophylline on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from control patients (CR) and patients with Crohn's disease (CD) and ulcerative colitis (UC). The drug was preincubated with cells for 10 min before challenge. The control releases were 17.2±4.3 %, n=4, CR; 18.6±3.9 %, n=3, CD and 12.4±0.6 %, n=3, UC.

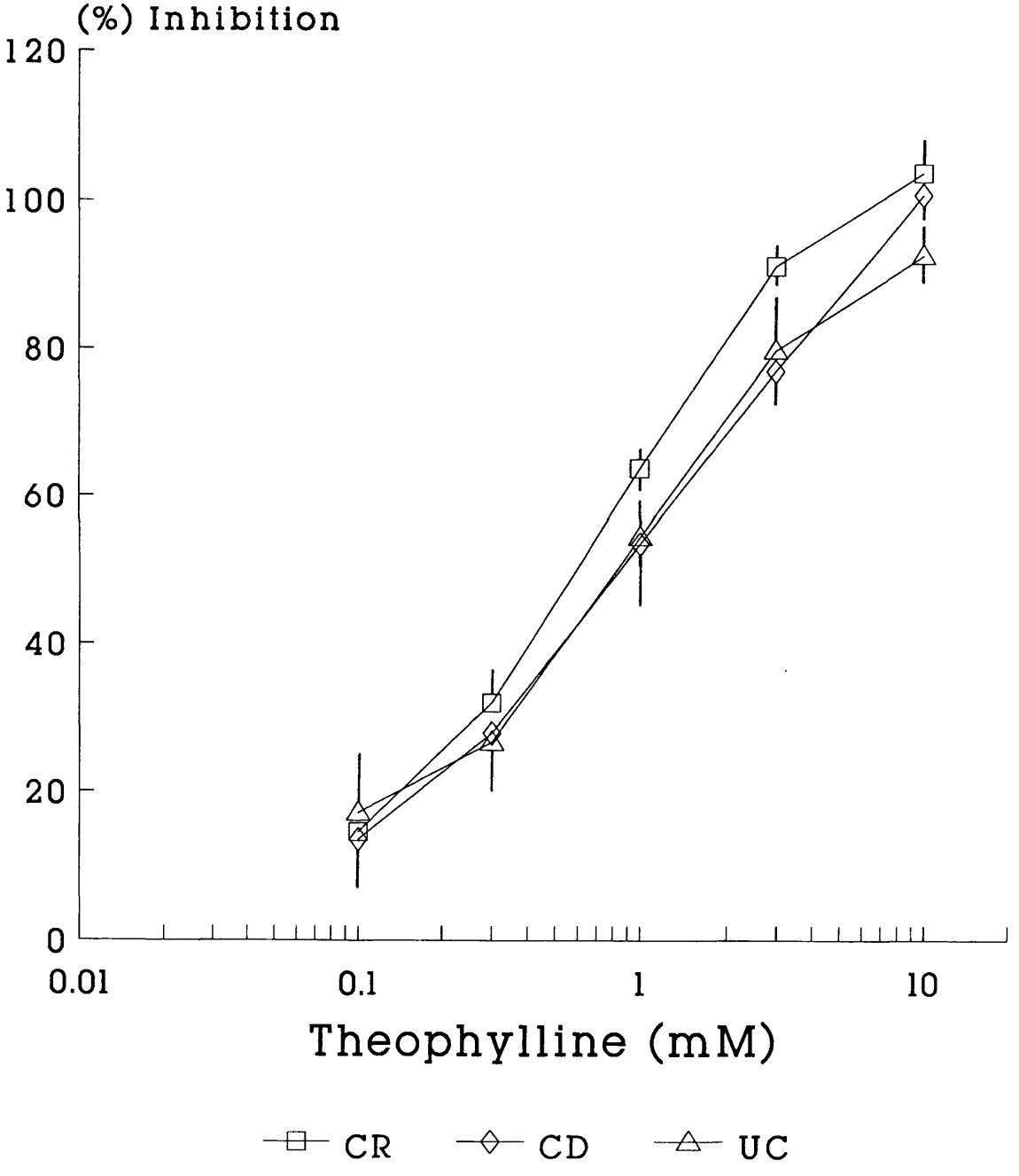


Fig 8.22 Effect of isoprenaline on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from control patients (CR) and patients with Crohn's disease (CD) and ulcerative colitis (UC). The drug was preincubated with cells for 10 min before challenge. The control releases were 17.4±3.7 %, n=5-6, CR; 17.1±3.8 %, n=3, CD and 23.5±11.1 %, n=3, UC.

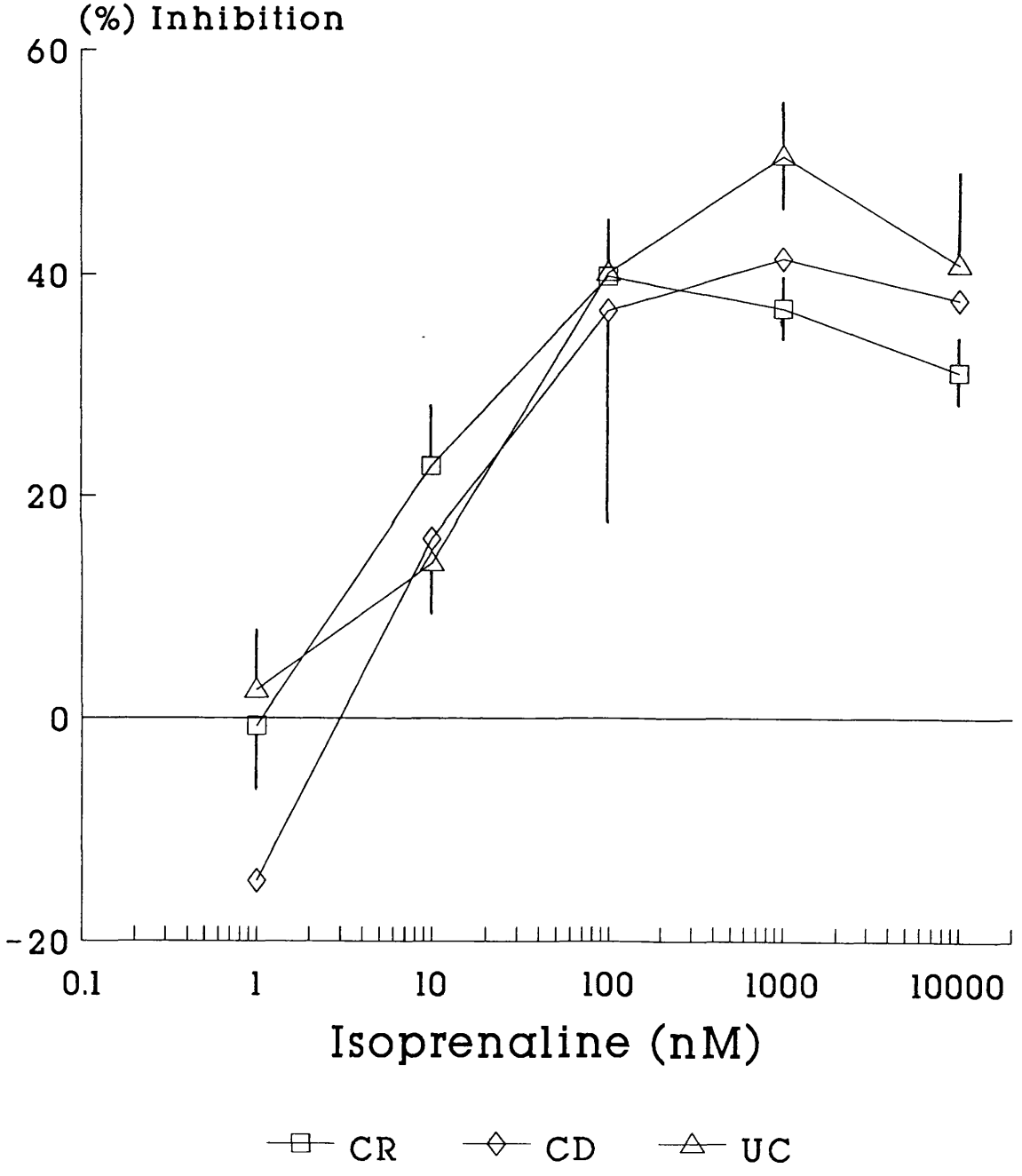


Fig 8.23 Effect of salbutamol on anti-IgE-induced histamine release from isolated human colonic mucosal mast cells from control patients (CR) and patients with Crohn's disease (CD) and ulcerative colitis (UC). The drug was preincubated with cells for 10 min before challenge. The control releases were 17.4±3.7 %, n=4-6, CR; 17.1±3.8 %, n=3, CD and 23.5±11.1 %, n=3, UC.

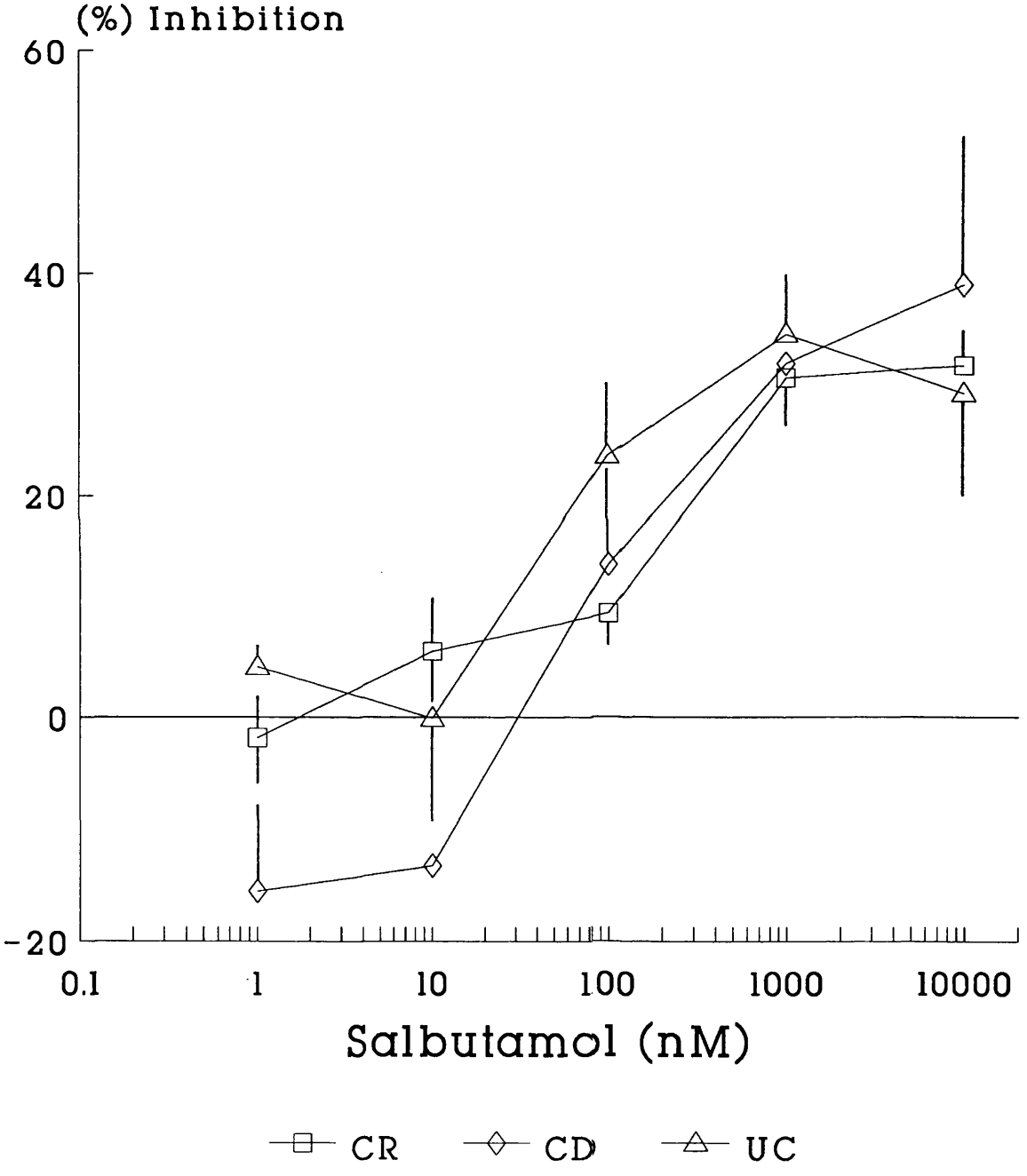


Table 8.1 Some basic properties of human colonic mucosal mast cells of control patients (CR) and patients with Crohn's disease (CD) and ulcerative colitis (UC).

	CR n=10	CD n=5	UC n=4
Dispersion efficiency (%)	18.0±1.2	29.9±3.7***	28.8±5.8***
Mast cells recovered x 10^6/g tissue	0.9±0.1	2.1±0.4***	2.2±0.6***
Estimated mast cells x 10^6/g tissue	5.4±1.0	6.8±0.8***	7.1±0.5***
Estimated histamine (µg/g tissue)	13.4±2.2	14.5±1.9	14.8±1.4
Mast cells (% total)	6.2±1.4	11.0±3.3	7.8±1.8
Viable cells (% total)	88.1±1.6	88.5±2.1	86.1±3.6
Spontaneous histamine release (%)	5.1±1.1	3.2±0.6	7.0±3.4
Histamine (pg/mast cell)	2.7±0.2	2.1±0.1***	2.1±0.2***
Formaldehyde sensitive (%)	91.3±1.3	80.0±2.7***	83.0±3.1***
Safranin positive (%)	0.0±0.0	0.0±0.0	0.0±0.0

** and ***: Denotes values which are significantly different ($P < 0.01$ and $P < 0.001$) from that of controls.

Table 8.2 Histamine release from human colonic mucosal mast cells of control patients (CR) and patients with Crohn's disease (CD) and ulcerative colitis (UC) induced by compound 48/80.

Concentration (μg/ml)	Histamine release (%) CR n=5	CD n=4	UC n=4
10	-0.6±0.8	2.1±0.3	2.5±0.8
1	-1.3±0.9	0.8±0.5	1.6±0.9
0.5	-1.1±0.6	0.5±0.6	0.7±0.4
0.25	-1.7±1.0	0.2±0.5	0.2±0.4
0.1	-1.7±0.9	0.3±0.2	0.1±0.2

Table 8.3 Histamine release from human colonic mucosal mast cells of control patients (CR) and patients with Crohn's disease (CD) and ulcerative colitis (UC) induced by substance P.

Concentration (μM)	Histamine release (%) CR n=5	CD n=4	UC n=4
50	2.7±1.1	3.7±0.5	3.3±0.7
10	0.1±1.2	1.2±0.3	2.5±0.5
5	-0.6±0.8	0.7±0.1	1.1±0.7
1	-0.9±0.9	0.1±0.3	0.5±0.3
0.1	-0.3±0.6	-0.1±0.3	-0.9±0.6

CHAPTER NINE

SOME STUDIES ON HUMAN MAST CELLS FROM THE STOMACH MUCOSA AND UTERINE MYOMETRIUM

9.1 Introduction

Mast cells are widely distributed in the human and animal uterus and are frequently found in the stratum vascularis of the myometrium, often in close proximity to blood vessels, and in the basal portion of the endometrium [408,409]. The numbers of mast cells in both sites vary with the oestrus cycle of the animal. Thus, they increase before ovulation, reaching a maximum during ovulation and thereafter drop sharply following implantation and attachment of the blastocyte [408,410,411]. The latter changes are paralleled by a fall in the histamine content of the uterus and correspond temporally with a rise in the level of circulating oestrogens. These hormones are thus believed to act as the stimulus for mast cell degranulation and the oestrogenic properties of the blastocyte have been described by many workers [408,411]. More recently, Cocchiara et al [412,413] have isolated a histamine releasing factor from the culture medium of human embryos (Embryo-derived histamine releasing factor: EHRF) which has subsequently been demonstrated to be capable of releasing histamine from enzymically dispersed rat uterine mast cells [414].

The degranulation of uterine mast cells as a result of blastocyte implantation would appear to indicate their involvement in the reproductive process. In support of this theory, treatment with antihistamines or mast cell degranulating agents may prevent decidua formation and implantation of the ovum in the rat [415,416]. The precise role of the mast cell in these processes is uncertain but the earliest changes following implantation closely resemble those of the inflammatory response [408], including a characteristic local increase in vascular permeability, and may variously involve the release of histamine, kinins, and prostaglandins [408], all of which are potentially derived from mast cells. Moreover, in addition to their possible role in implantation, the mast cell may also be involved in various forms of uterine dysfunction [409].

Abnormal bleeding following the introduction of intrauterine contraceptive devices (IUD) is a major disadvantage of these appliances and is connected with an elevation in the number and degranulation of local uterine mast cells [417]. These cells may also be involved in other common disorders of menstruation, including both excess bleeding and dysmenorrhoea [409].

The control of acid secretion by the stomach is complex and is regulated by chemical signals delivered by neurocrine, endocrine and paracrine routes [418]. In essence, gastrin, acetylcholine and histamine act in concert to produce the release of acid from the gastric parietal cell. All vertebrate species capable of secreting acid have high levels of histamine in the stomach. However, the exact localisation of the amine varies according to the species. In the rat, histamine is found predominantly in enterochromaffin-like (ECL) cells while in the dog and in man, it is confined largely to gastric mast cells, located in close proximity to parietal cells [419-421].

Two theories have been proposed for the cellular control of gastric acid secretion. The permissive theory [422] proposed that gastrin, acetylcholine and histamine all act on individual receptors on the parietal cell, with the effect of the former two substances being potentiated, or dependent upon, the additional binding of histamine. The transmission theory [422], in contrast, suggested that histamine is the final common activator for secretion and that gastrin and acetylcholine act on the mast cell to cause histamine release, which in turn activates the parietal cell. Since their initial proposal, the permissive theory has been given much greater recognition but recently, Black and Shankley [422] suggested that the transmission theory may provide a much more convincing and detailed explanation of the currently available data. In contrast, Soll et al [421] have demonstrated that gastrin and acetylcholine both failed to release histamine from enzymically dispersed canine fundic mast cells. However, these authors point out that their data must be interpreted with extreme caution and that great care must be taken in extrapolating from one species to another.

Despite these different views, the role played by histamine in the control of gastric acid secretion is indisputable. Indeed, the efficacy of H_2-antagonists in the treatment of peptic ulcer disease further underlines the therapeutic importance of this

effect. Moreover, a variety of mast cells stabilizing drugs including DSCG and the benzopyran 2-carboxylic acid FPL 52694 inhibit acid secretion, and prevent experimentally induced gastric lesions, in both man and experimental animals [423-427]. With this in mind, it is the aim of the present study to isolate mast cells from the human stomach, the main source of gastric histamine in man, as well as those from the uterine myometrium. These cells are then characterized in terms of their basic histochemical and functional properties. Such an investigation should ultimately provide some insight into the role of the uterine mast cell in implantation and reproduction, and of the gastric mast cell in acid secretion and peptic ulcer disease. Moreover, these data should also provide further information on the extent of mast cell heterogeneity in man.

9.2 Methods

All methods used in this study were as described in chapter two.

9.3 Results

Unless otherwise stated, histamine release in experiments involving anti-allergic compounds were induced by anti-human IgE.

9.3.1 Basic properties of human stomach mucosal and uterine myometrial mast cells

Using an identical isolation method, free cell suspensions were obtained from both stomach and uterine tissues. The enzymic dispersion yielded $0.8\pm0.2 \times 10^6$ (stomach mucosa) and $0.2\pm0.1 \times 10^6$ (uterine myometrium) mast cells per gram wet tissue (n=4-7) (table 9.1). Mast cells comprised 4.8±0.8 and 3.1±0.7 % (n=4-7) of the total nucleated cells. The histamine contents per mast cell were 2.3±0.2 and 1.8±0.5 pg (n=4-7) respectively.

Cells obtained from the two locations were structurally intact as judged under light microscopy, highly viable and exhibited low spontaneous histamine release (table 9.1). Moreover, mast cells obtained from the stomach and uterine myometrium were

predominantly sensitive to formaldehyde fixation.

9.3.2 Functional properties of human stomach mucosal mast cells

Isolated mast cells from the human stomach mucosa released histamine in a concentration-related fashion in response to stimulation with anti-IgE (fig 9.1a) and concanavalin A (fig 9.1b). These cells were also responsive towards the calcium ionophores A23187 (fig 9.2a) and ionomycin (fig 9.2b) but were essentially refractory or only very weakly sensitive to the polybasic agents compound 48/80, polymyxin, substance P, $SP_{1-4}C_{12}$, polylysine and polyarginine (tables 9.2-9.7). In preliminary experiments (data not shown), isolated stomach mucosal mast cells failed to respond to both gastrin and acetylcholine.

The anti-allergic drugs DSCG (fig 9.3) and nedocromil sodium (fig 9.4) both dose-dependently inhibited anti-IgE-induced histamine release from human stomach mucosal mast cells; nedocromil sodium was at least one order of magnitude more active than DSCG. Both agents, however, at the highest concentration tested, exhibited tachyphylaxis after a 10 minutes preincubation with the cells before challenge (figs 9.3 and 9.4). The phosphodiesterase inhibitor theophylline was found to suppress completely histamine release induced by anti-IgE (fig 9.5) while the inhibitory effects produced by the β-adrenoceptor agonists isoprenaline (fig 9.6) and salbutamol (fig 9.7) were observed to be much weaker.

9.3.3 Functional properties of human uterine myometrial mast cells

Similarly to the stomach mast cell, those derived from the uterine myometrium released histamine on challenge with anti-IgE (fig 9.8a), concanavalin A (fig 9.8b), A23187 (fig 9.9a) and ionomycin (fig 9.9b) but were essentially unresponsive to compound 48/80, polymyxin, substance P, $SP_{1-4}C_{12}$, polylysine and polyarginine (tables 9.2-9.7).

In preliminary experiments involving anti-allergic compounds, both DSCG (fig 9.10) and nedocromil sodium (fig 9.11) inhibited anti-IgE-induced histamine release

from mast cells from the uterine myometrium, with a maximum inhibition of around 30 %. Moreover, unlike the stomach cell, tachyphylaxis was not observed and the inhibition was maintained with preincubation (figs 9.10 and 9.11). In further contrast to the stomach cell, both isoprenaline (fig 9.13) and salbutamol (fig 9.14) were found to have potent inhibitory activity; maximum inhibition achieved by both β-adrenoceptor agonists was comparable to that observed for theophylline (fig 9.12).

9.4 Discussion

The present study has demonstrated that functional human mast cells may be obtained by the enzymic dispersion of the stomach mucosa and uterine myometrium. Isolated cells from the two locations were highly viable, structurally intact as judged under light microscopy and exhibited a low spontaneous release of histamine. Moreover, these cells have been characterized in terms of their basic histochemical and functional properties.

Isolated mast cells from both the stomach mucosa and uterine myometrium were predominantly sensitive to formaldehyde fixation and, in this respect, they are similar to those found in the lung parenchyma and colonic mucosa. Moreover, initial study has shown that the two mast cell preparations can be stained with alcian blue but cannot be counterstained with safranin. These findings have again highlighted the danger in the casual usage of the terms mucosal mast cell(s) (MMC) and connective tissue mast cell(s) (CTMC). Indeed, by anatomical definitions, the stomach mucosal mast cell should belong to the former category whereas the uterine myometrial mast cell is clearly more related to the latter category. However, the present study has demonstrated that the two cell preparations are undoubtedly similar in their histochemical properties.

In terms of their responses towards a number of immunological and non-immunological secretagogues, isolated mast cells from the stomach mucosa and uterine myometrium also exhibited similar functional properties. Both cell preparations released histamine in response to stimulation with IgE-directed ligands and calcium ionophores but were essentially insensitive to polybasic agents and neuropeptides. These functional characteristics are again similar to those observed for mast cells from the lung and

colon [76,280,281], but differ from those of the cells derived from the skin [66,277,309], thereby further confirming the unique character of the cutaneous mast cell.

The role of the uterine mast cell and of histamine in implantation, the formation of a decidual cell response, gestation, and parturition has been the subject of debate for many years and is still the source of dispute. However, from animal studies [408,410,411], it is now widely recognised that implantation and attachment of the blastocyte is normally accompanied by degranulation of local uterine mast cells. Recently, Cocchiara et al [412,413] have isolated a histamine releasing factor from the culture medium of human embryos (EHRF) which they proposed to be capable of inducing the release of histamine from uterine mast cells at the time of implantation [428]. They further suggested that the secreted histamine, either alone or in cooperation with other factors and/or mechanisms, may play a role in preventing immune rejection of the embryo and in the control of vascular permeability at the implantation stage. To substantiate their hypothesis, these authors isolated mast cells from the rat uterus and found that EHRF was capable of triggering histamine release from these cells but not from those of the peritoneal cavity [414]. However, it must be noted that these isolated rat uterine mast cells also responded to compound 48/80, a property that is not shared by their corresponding human counterparts as demonstrated in the present study. This finding not only illustrates the existence of mast cell heterogeneity between different species but also suggests that the rat uterine mast cell may not be the ideal model from which to extrapolate to the situation in the human. With this in mind, it would be of interest and also of great importance to see whether EHRF could produce a similar effect on isolated human uterine mast cells.

The role of histamine in the stimulation of acid secretion by gastric parietal cells has been well documented. Moreover, the importance of this effect is reflected in the clinical utility of H_2-receptor antagonists in the treatment of peptic ulcer disease. With this in mind, two theories have been proposed to account for the action of histamine [422,429]. The permissive theory suggested that the gastric parietal cell has specific receptors for gastrin, acetylcholine and histamine, with the effect of the former two substances being potentiated, or dependent upon, the additional binding of the biogenic amine. The transmission theory, in contrast, proposed that histamine is the final

common trigger for secretion and that gastrin and acetylcholine act on the gastric mast cell to cause histamine release, which in turn activates the parietal cell. Since its initial proposal, the latter theory has fallen into neglect but has recently been forcibly resurrected by Black and Shankley [422] who consider that it provides a much more convincing and detailed explanation of the currently available data. However, in an attempt to clarify this problem, Soll et al [421] isolated mast cells from canine fundic mucosa but found that neither acetylcholine nor gastrin caused histamine release. In contrast to the canine fundic mast cell, it has been shown that in both rabbit and frog gastric mucosa, where histamine is located predominantly in endocrine-like cells, both acetylcholine and gastrin are capable of inducing the release of this biogenic amine in vitro [430,431]. In the present study, preliminary experiments (data not shown) have indicated that dispersed human stomach mucosal mast cells, similarly to those from the canine fundic mucosa, are refractory to the effect of both acetylcholine and gastrin. These findings have again questioned the validity of the transmission theory but more detailed studies have to be carried out before definitive conclusions can be drawn.

Despite the confusion, the role played by histamine in the regulation of gastric acid secretion cannot be doubted especially in view of the efficacy of H_2-receptor antagonists in the treatment of peptic ulcer disease. With this in mind, and with the knowledge that histamine in the human stomach is confined almost entirely to gastric mast cells, stabilizing drugs against these cells may be of therapeutic value in the management of various gastric inflammatory disorders. In the present study, both DSCG and nedocromil sodium were capable of inhibiting anti-IgE-induced histamine release from isolated stomach mucosal mast cells; maximum inhibition was comparable with that observed for their lung and colonic counterparts. Interestingly, in in vivo studies, both DSCG and the benzopyran 2-carboxylic acid FPL 52694 have been shown to inhibit acid secretion, and to prevent experimentally induced gastric lesions in both man and experimental animals [423-427], thus indicating the possible clinical potential of these drugs. In preliminary experiments, immunologically-induced histamine release from isolated uterine myometrial mast cells was also similarly inhibited by the chromones.

Finally, similarly to those from the colon and lung, isolated mast cells from the stomach mucosa and uterine myometrium were subject to modulation by cAMP-active

compounds. In the former case, the phosphodiesterase inhibitor theophylline was much more potent than the β-adrenoceptor agonists isoprenaline and salbutamol in suppressing anti-IgE-induced histamine release. In contrast, in the uterine cell, maximum inhibition achieved by the two β-adrenoceptor agonists was comparable to that observed for theophylline. The reasons for this difference are not obvious but it may indicate that in the stomach mucosal mast cell, but not in the uterine myometrial mast cell, a large fraction of the immunologically released histamine is insulated from β-adrenergic modulation.

In total, the present study shows that mast cells from the human stomach mucosa and uterine myometrium, apart from some subtle differences, are in many respects both functionally and histochemically similar to their lung and colonic counterparts. Clearly, mast cell heterogeneity in man is much less sharply defined than in the rodent.

Fig 9.1 Histamine release induced by (a) anti-human IgE (n=7) and (b) concanavalin A (n=4) from isolated human stomach mucosal mast cells.

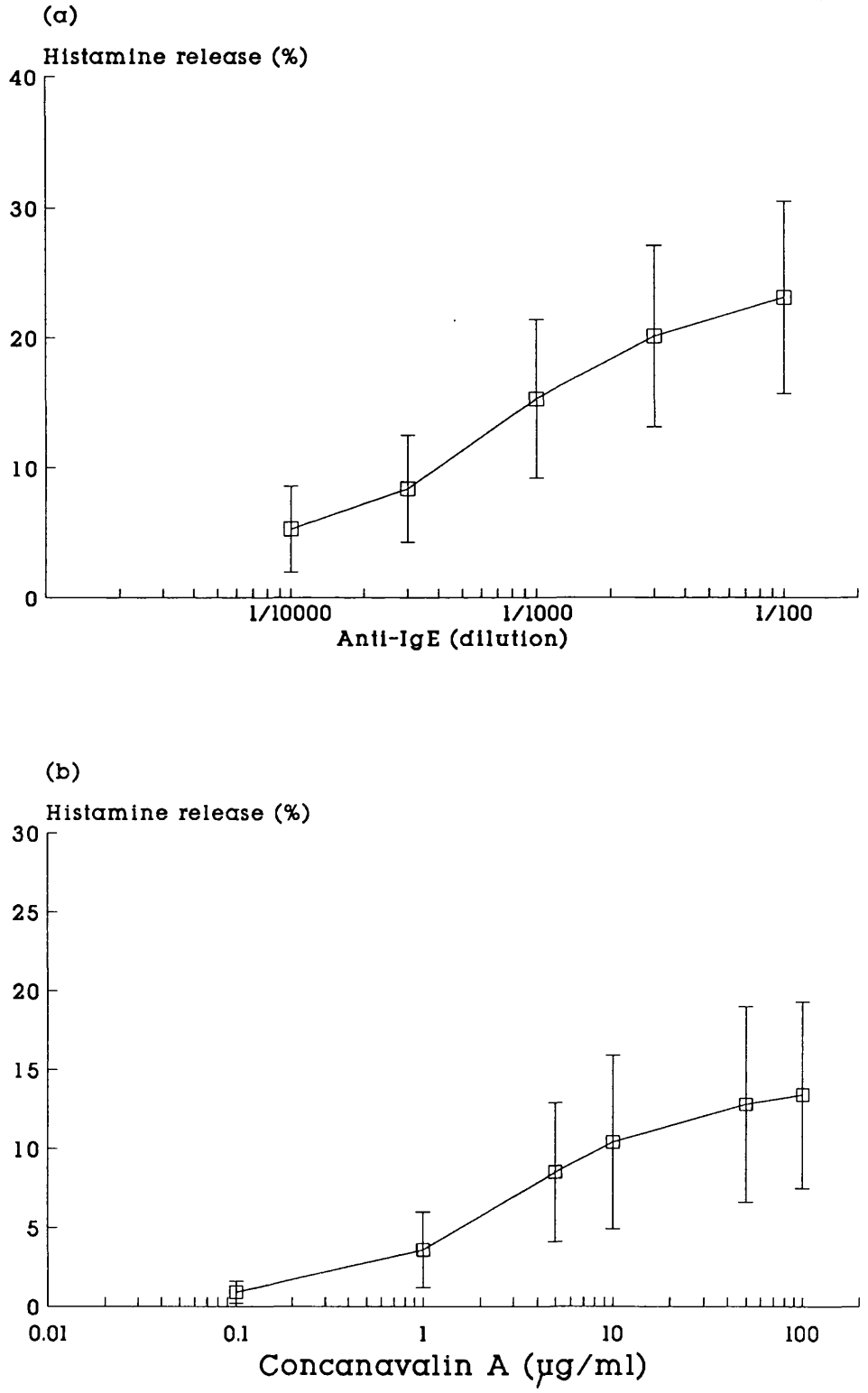
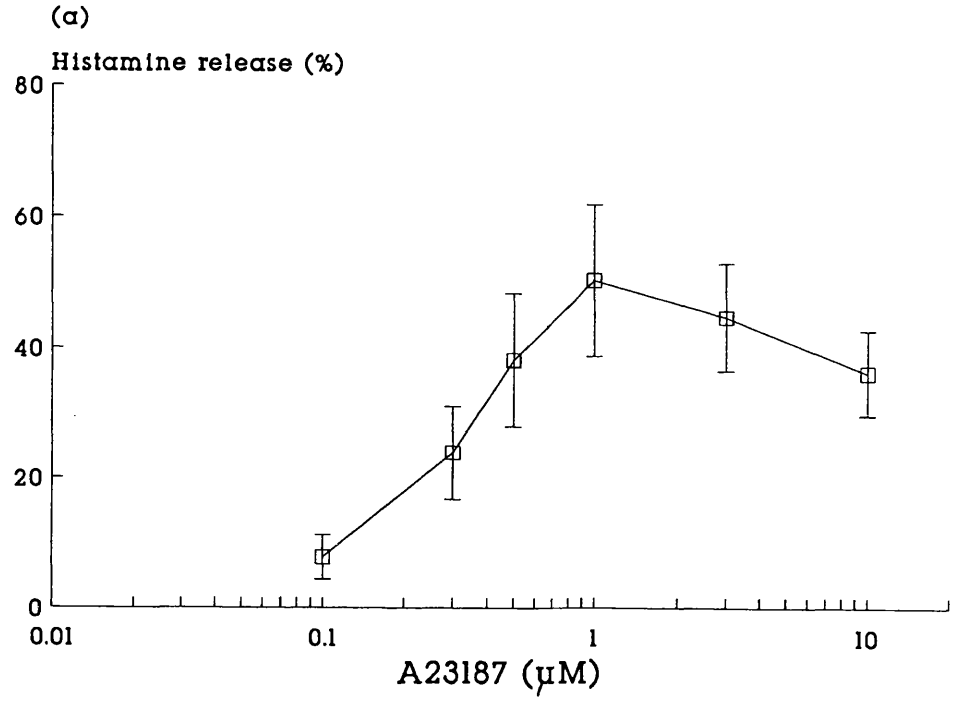


Fig 9.2 Histamine release induced by (a) A23187 (n=4) and (b) ionomycin (n=4) from isolated human stomach mucosal mast cells.



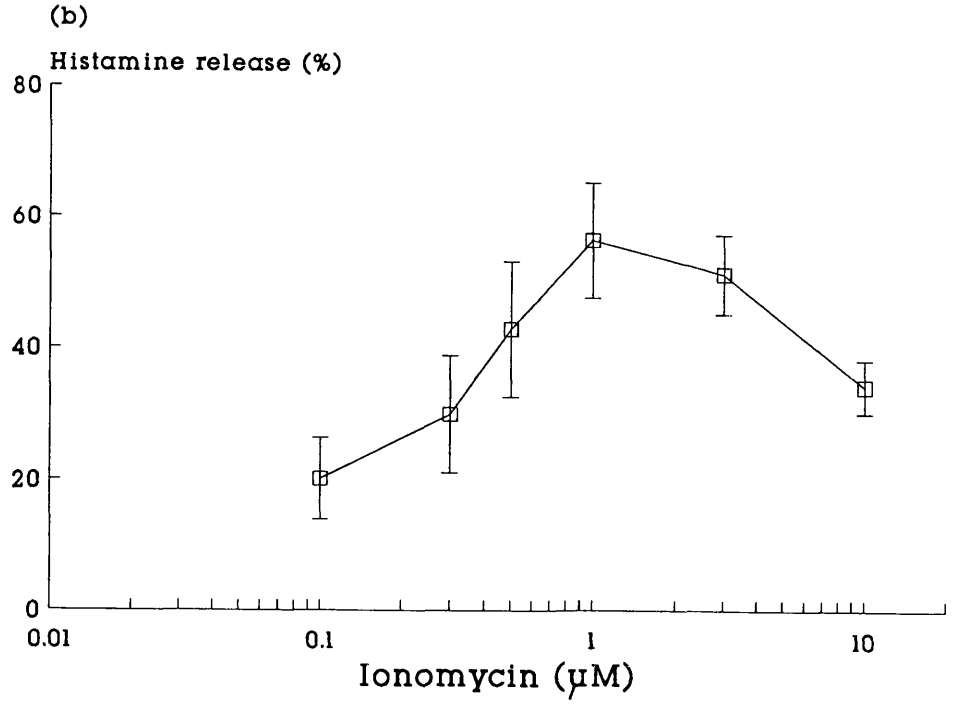


Fig 9.3 Effect of disodium cromoglycate (DSCG) on anti-IgE-induced histamine release from isolated human stomach mucosal mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 16.4±3.2 % (0') and 17.6±3.1 % (10') respectively, n=4, paired data.

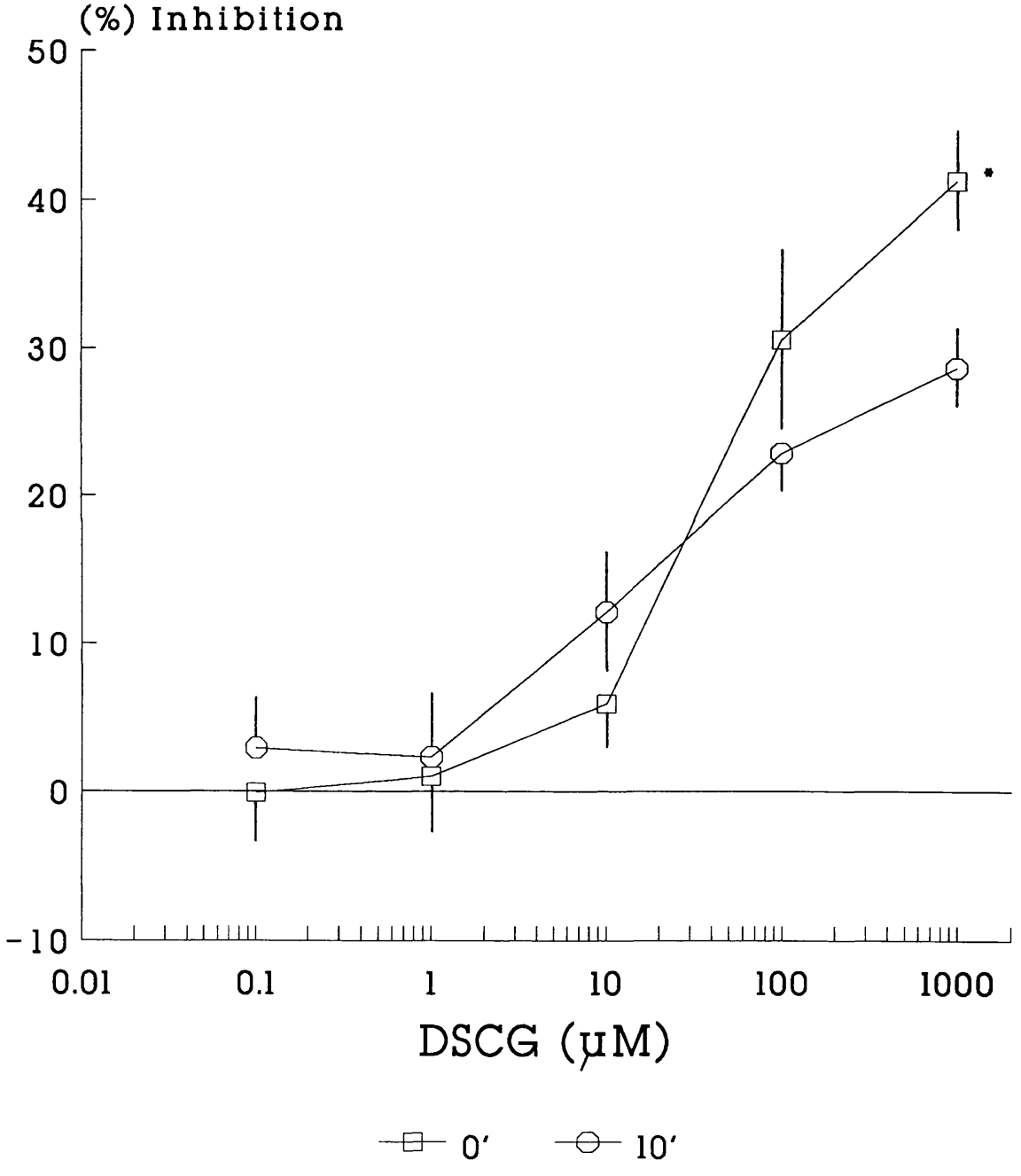


Fig 9.4 Effect of nedocromil sodium on anti-IgE-induced histamine release from isolated human stomach mucosal mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 16.4±3.2 % (0') and 17.6±3.1 % (10') respectively, n=4, paired data.

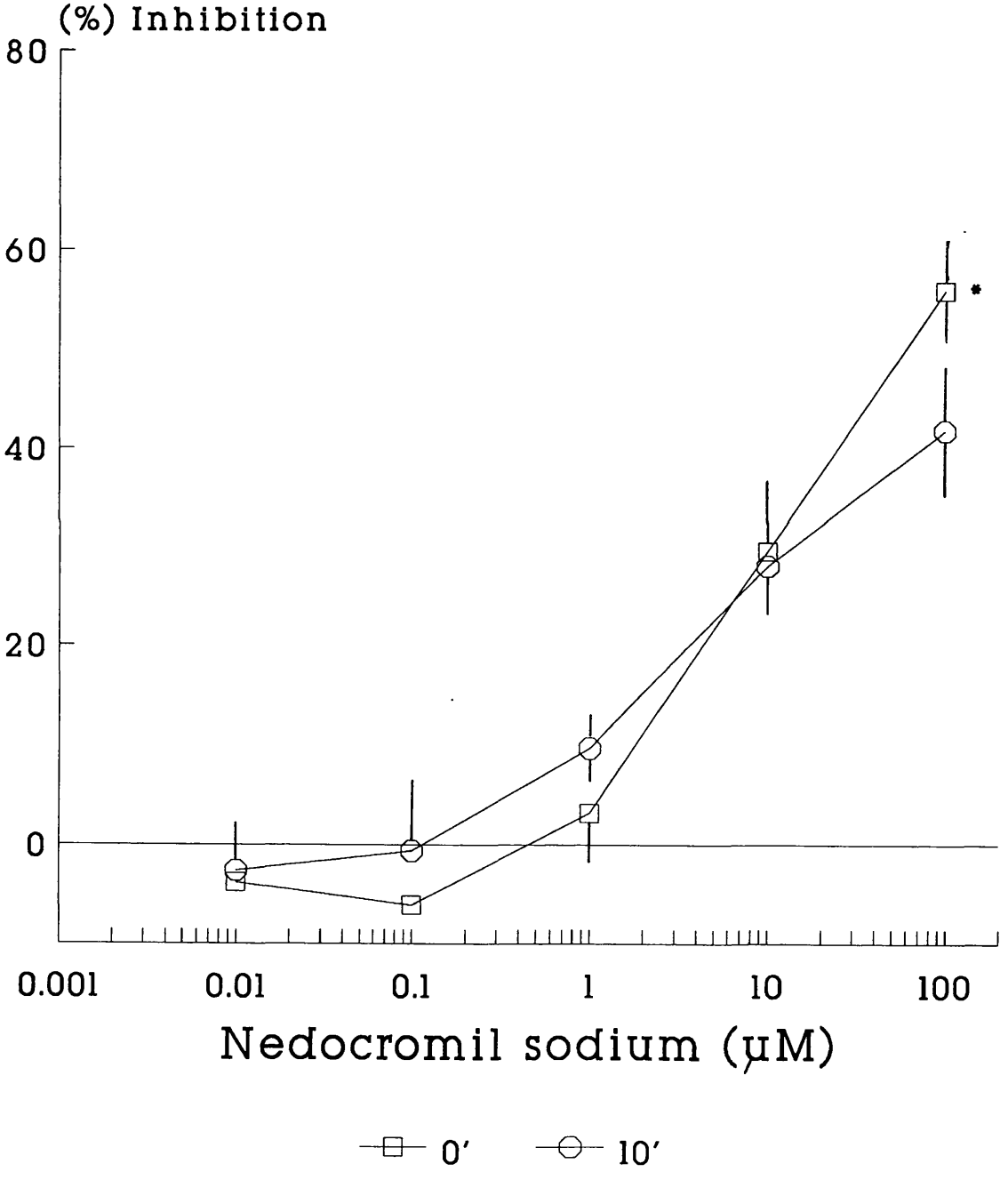


Fig 9.5 Effect of theophylline on anti-IgE-induced histamine release from isolated human stomach mucosal mast cells. The drug was preincubated with cells for 10 min before challenge. The control release was 15.8±3.7 %, n=4.

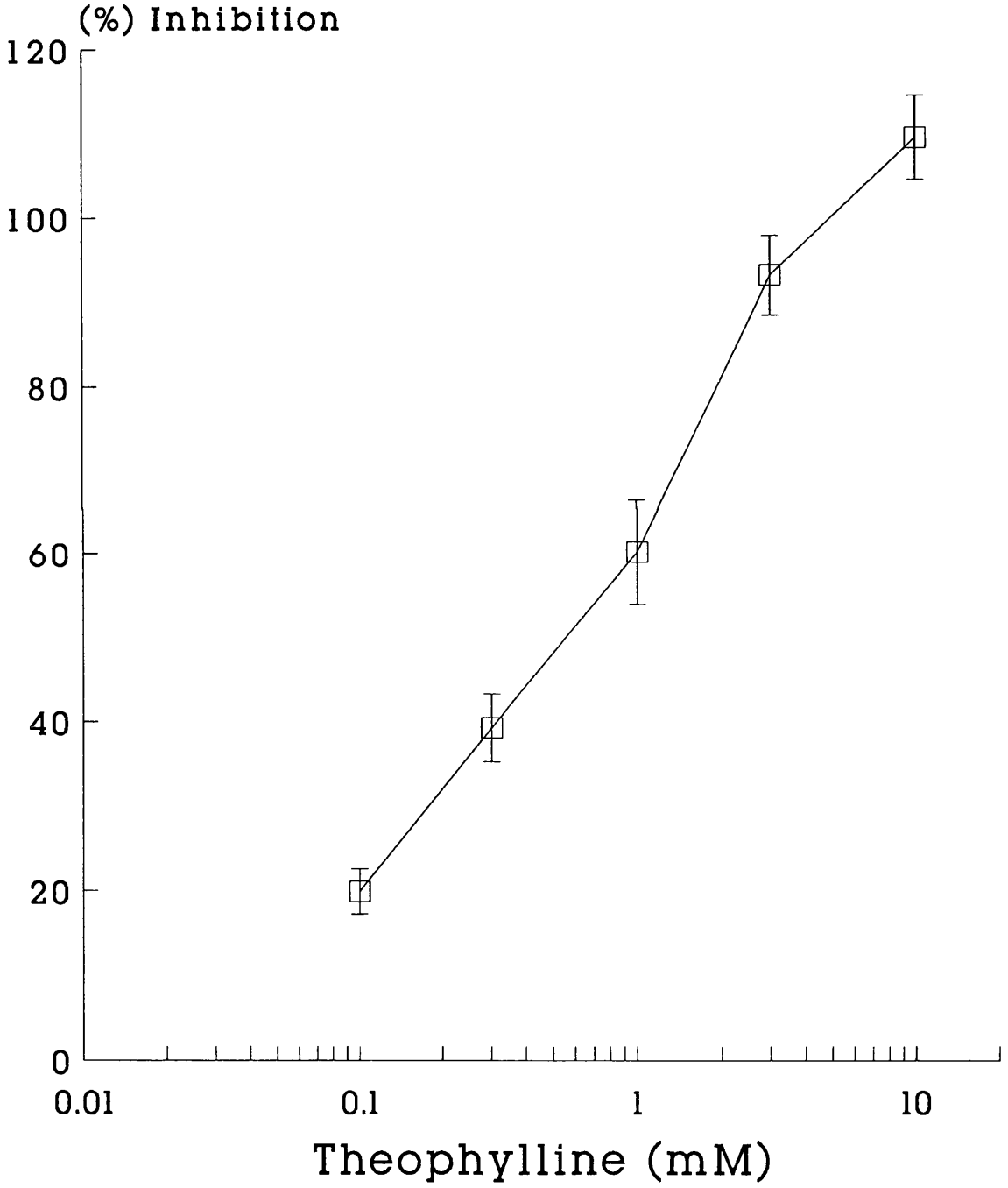


Fig 9.6 Effect of isoprenaline on anti-IgE-induced histamine release from isolated human stomach mucosal mast cells. The drug was preincubated with cells for 10 min before challenge. The control release was 19.3±3.5 %, n=3.

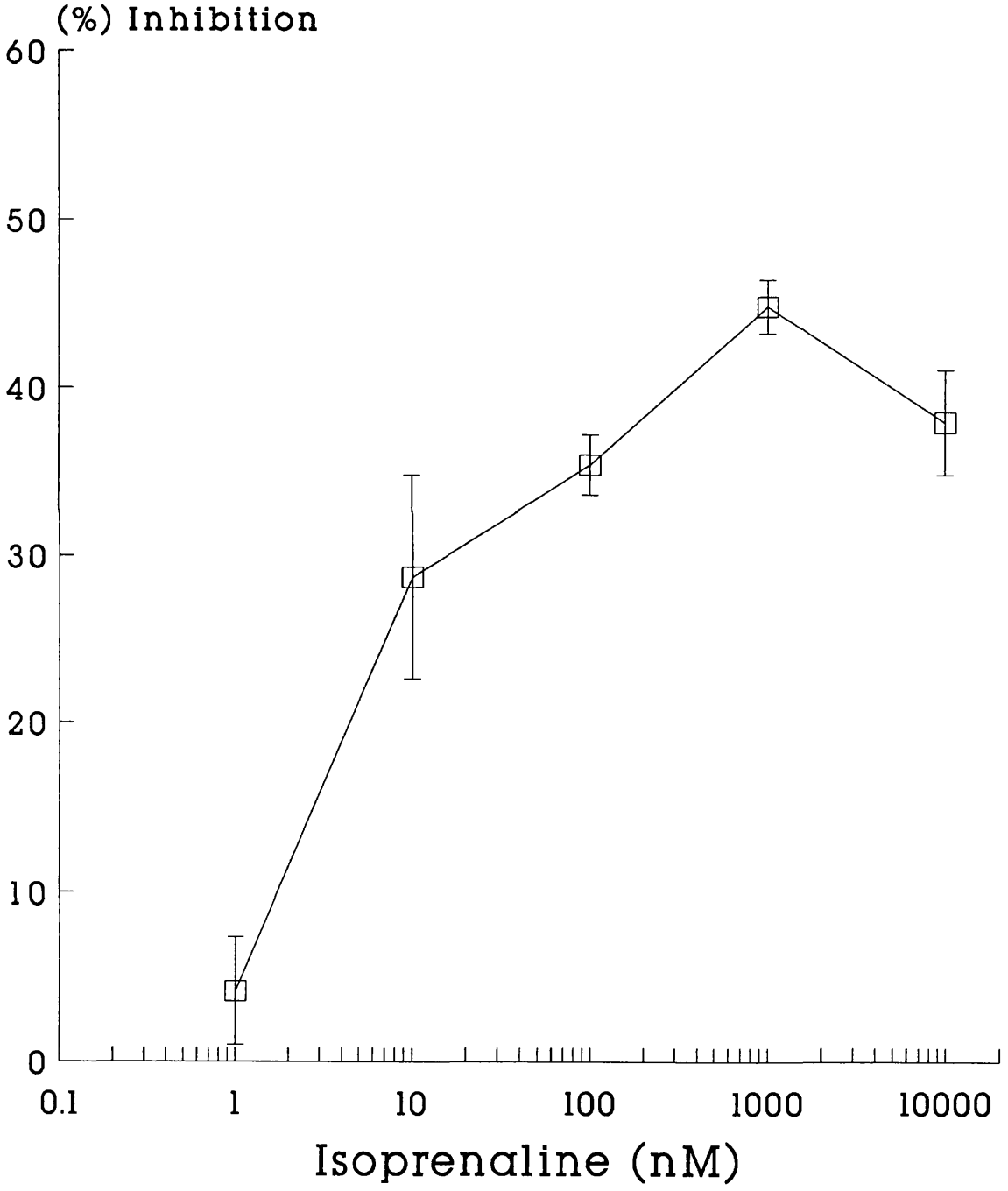


Fig 9.7 Effect of salbutamol on anti-IgE-induced histamine release from isolated human stomach mucosal mast cells. The drug was preincubated with cells for 10 min before challenge. The control release was 16.7±3.5 %, n=4.

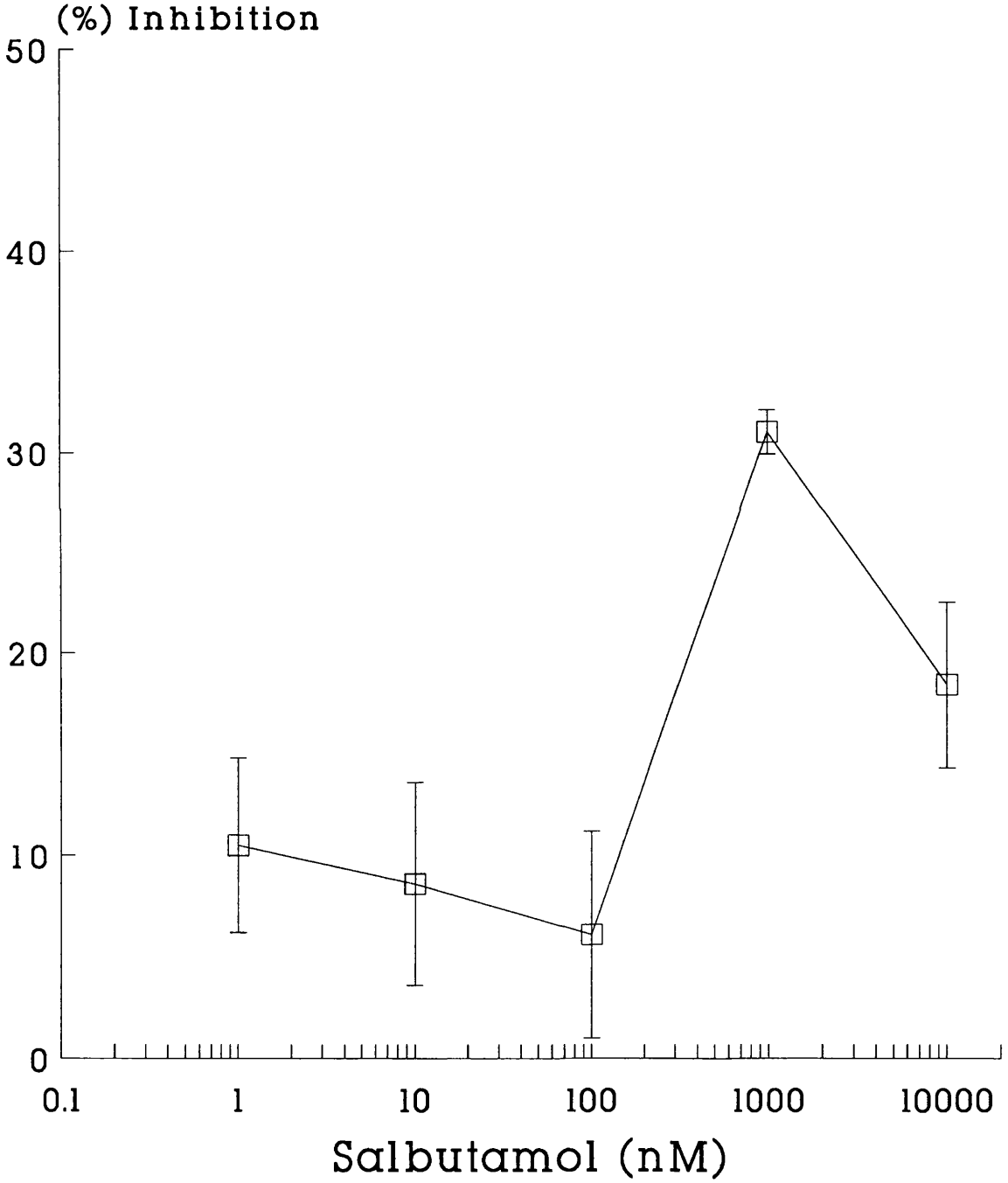
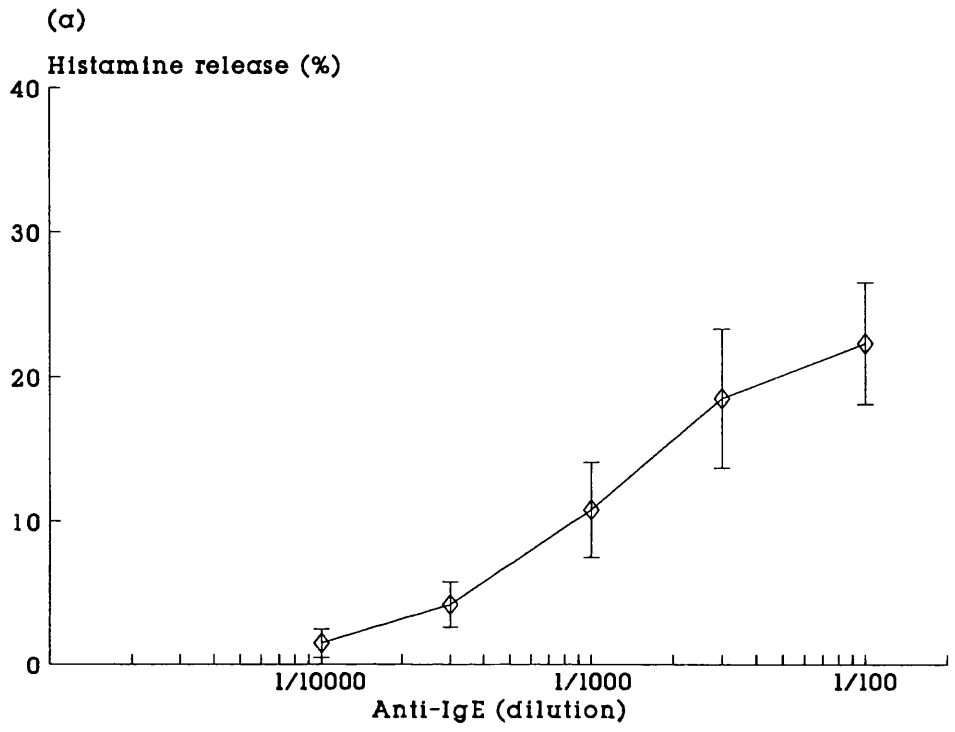


Fig 9.8 Histamine release induced by (a) anti-human IgE (n=6-9) and (b) concanavalin A (n=3) from isolated human uterine myometrial mast cells.



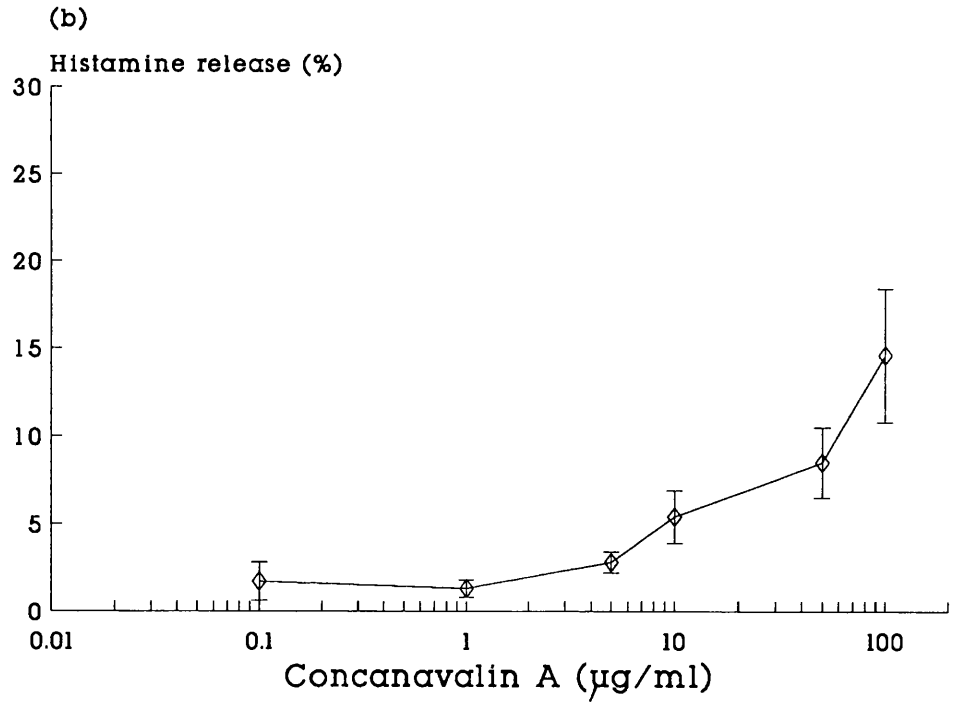
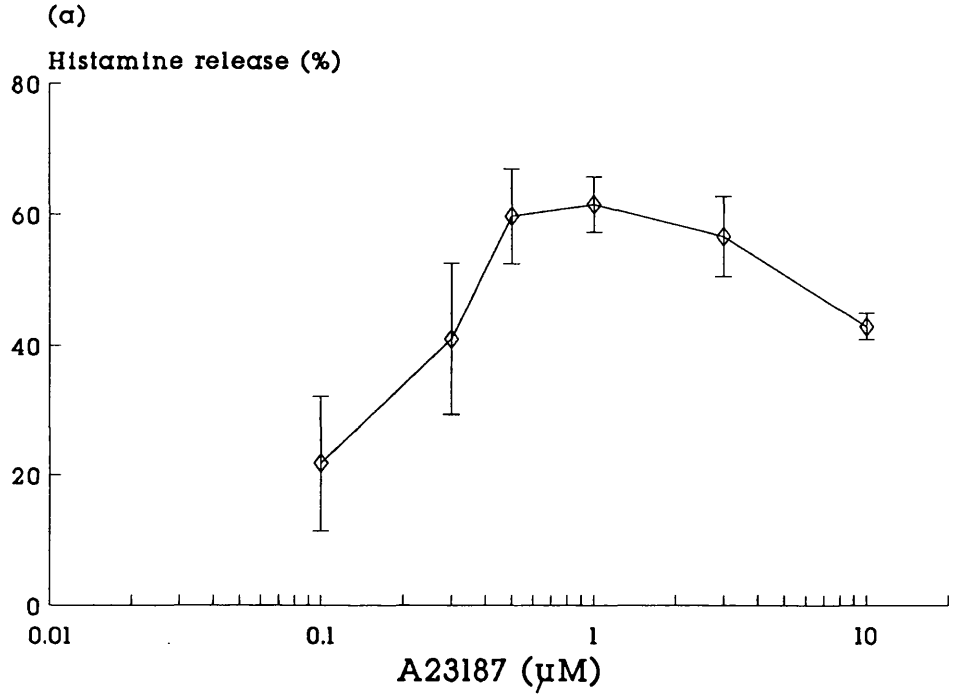


Fig 9.9 Histamine release induced by (a) A23187 (n=4) and (b) ionomycin (n=5) from isolated human uterine myometrial mast cells.



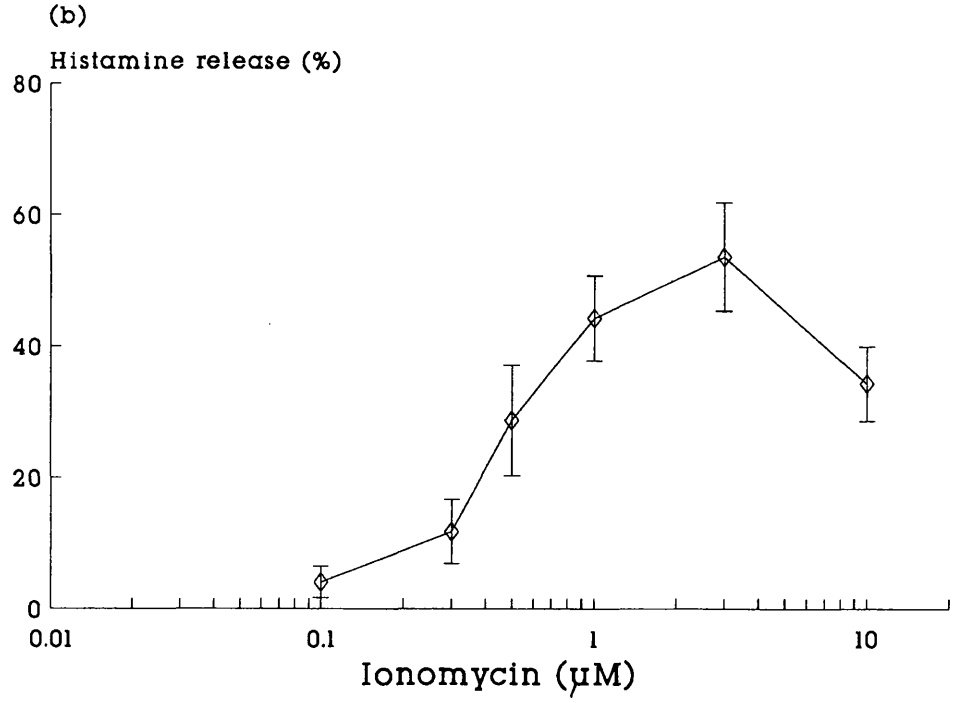


Fig 9.10 Effect of disodium cromoglycate (DSCG) on anti-IgE-induced histamine release from isolated human uterine myometrial mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 16.1 % (0') and 16.0 % (10') respectively, n=2, paired data.

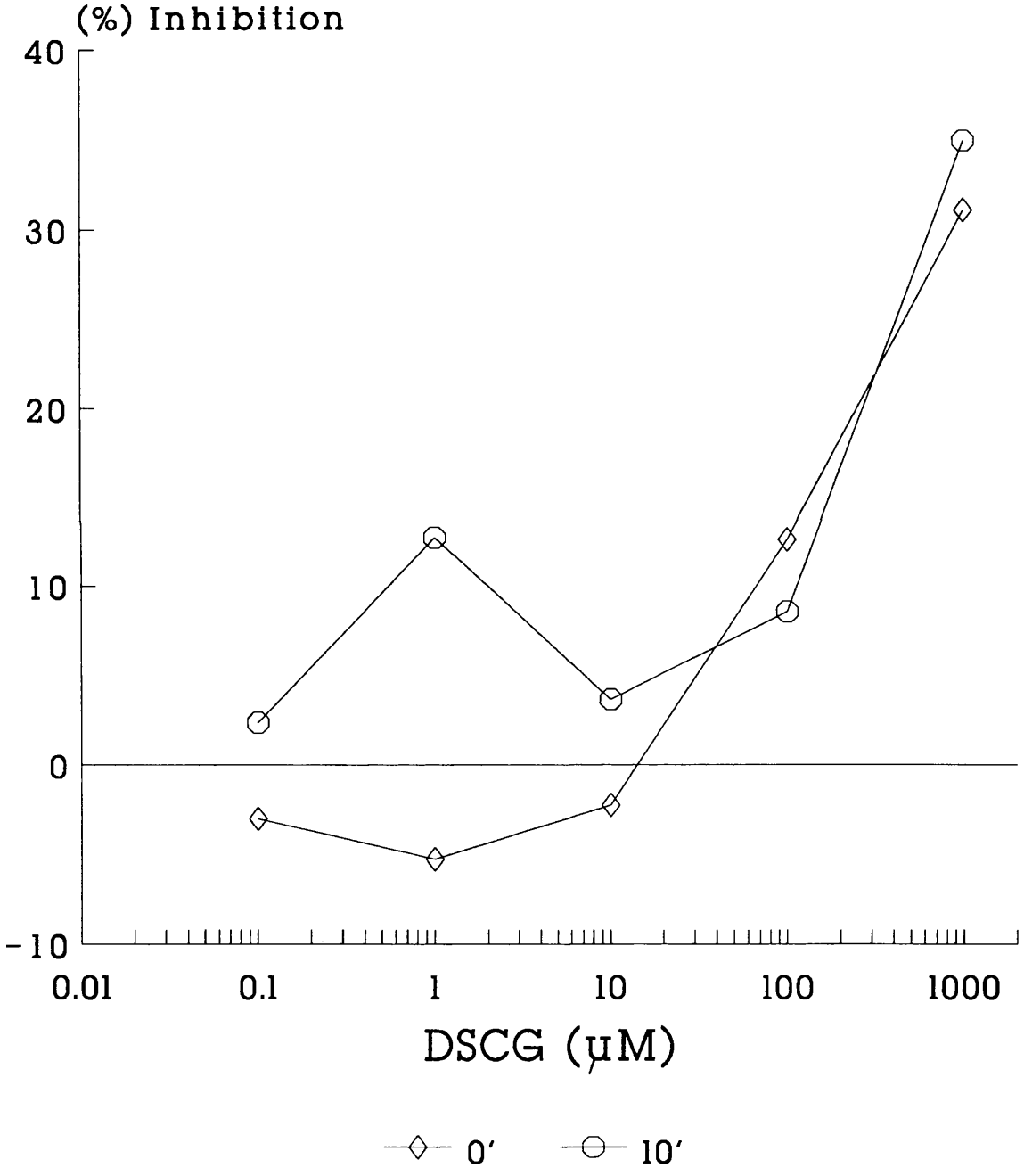


Fig 9.11 Effect of nedocromil sodium on anti-IgE-induced histamine release from isolated human uterine myometrial mast cells. The drug was preincubated with cells for 10 min (10') or added simultaneously with the stimulus (0'). The control releases were 16.1 % (0') and 16.0 % (10') respectively, n=2, paired data.

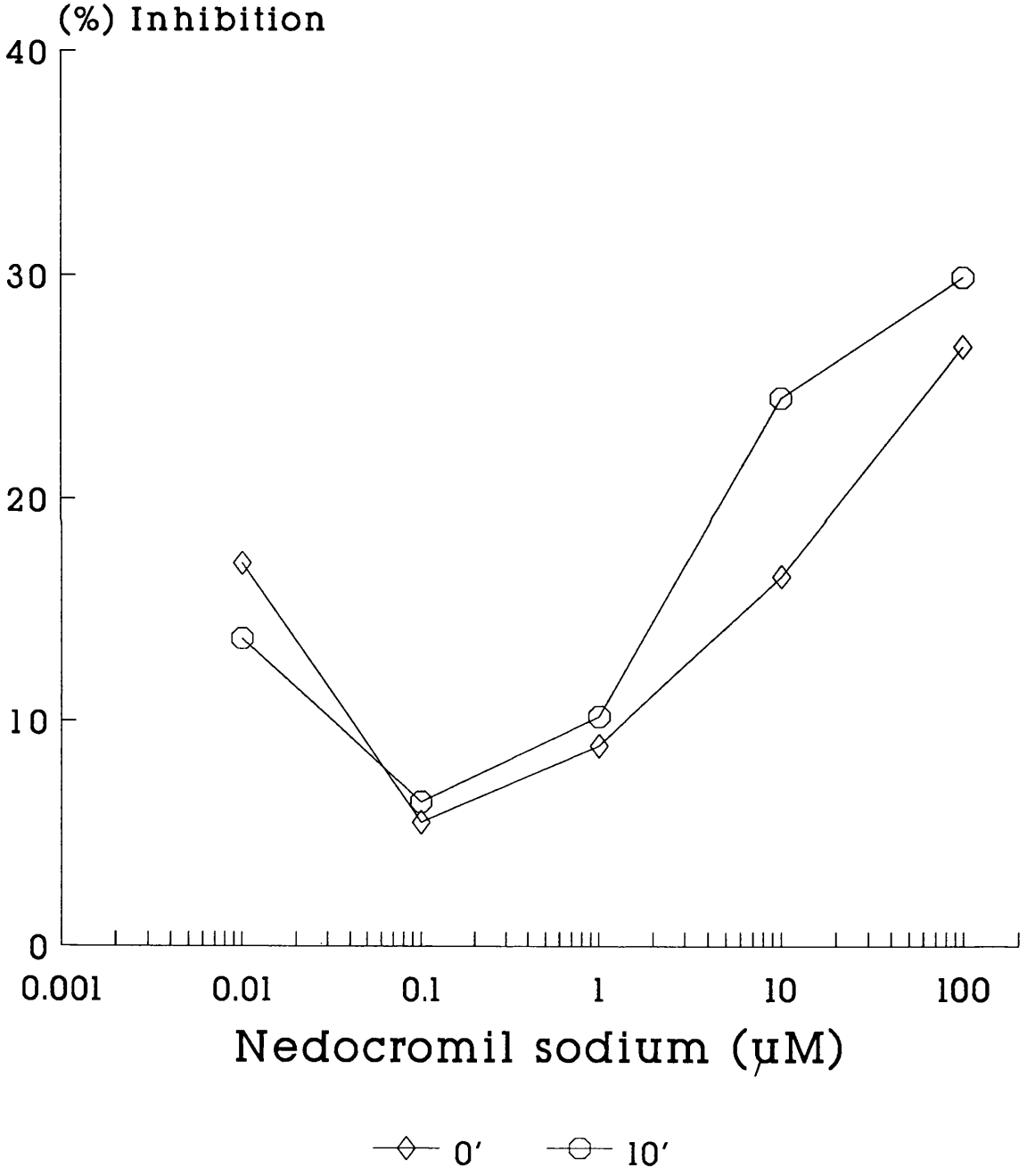


Fig 9.12 Effect of theophylline on anti-IgE-induced histamine release from isolated human uterine myometrial mast cells. The drug was preincubated with cells for 10 min before challenge. The control release was 9.7 %, n=1.

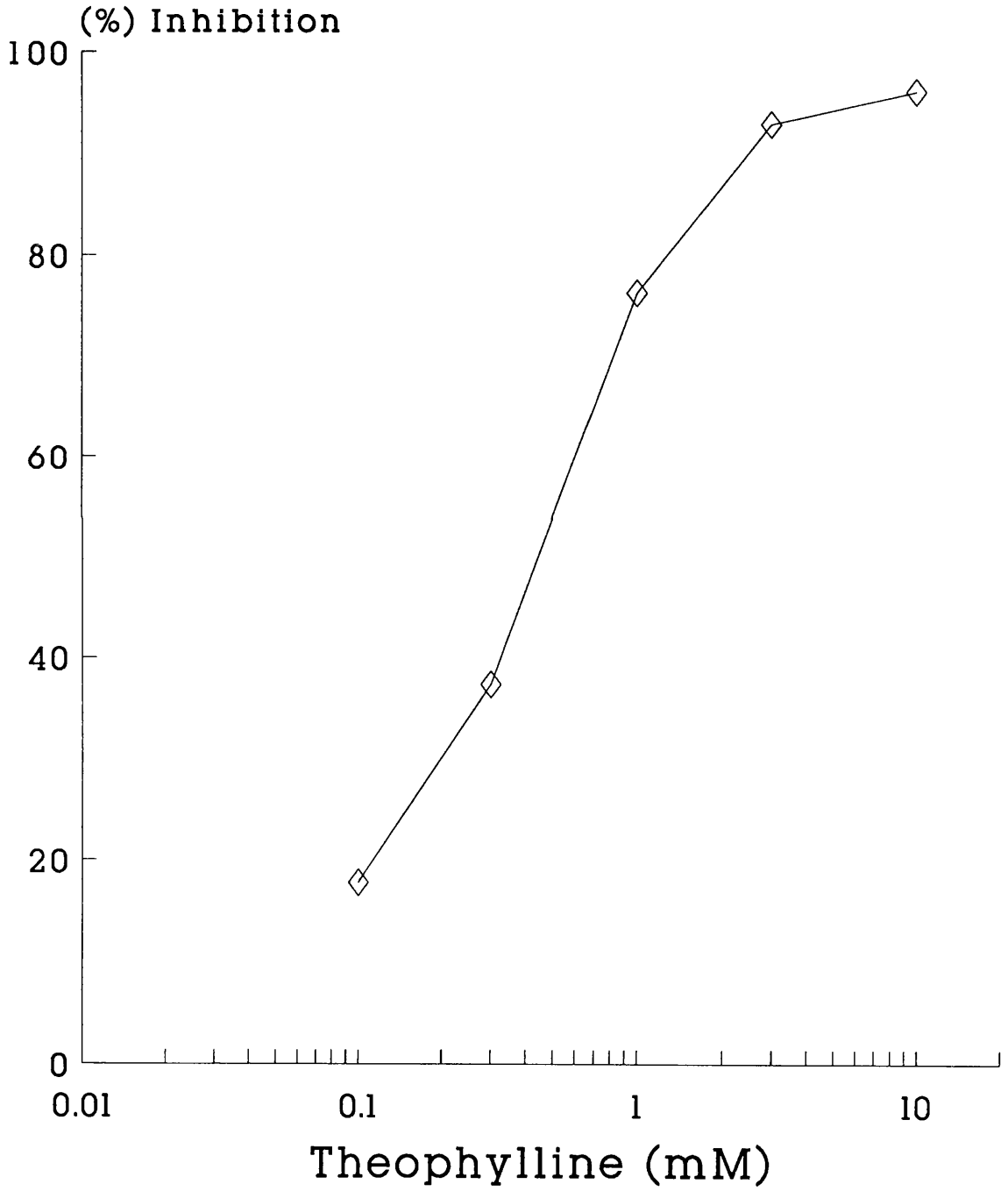


Fig 9.13 Effect of isoprenaline on anti-IgE-induced histamine release from isolated human uterine myometrial mast cells. The drug was preincubated with cells for 10 min before challenge. The control release was 9.7 %, n=1.

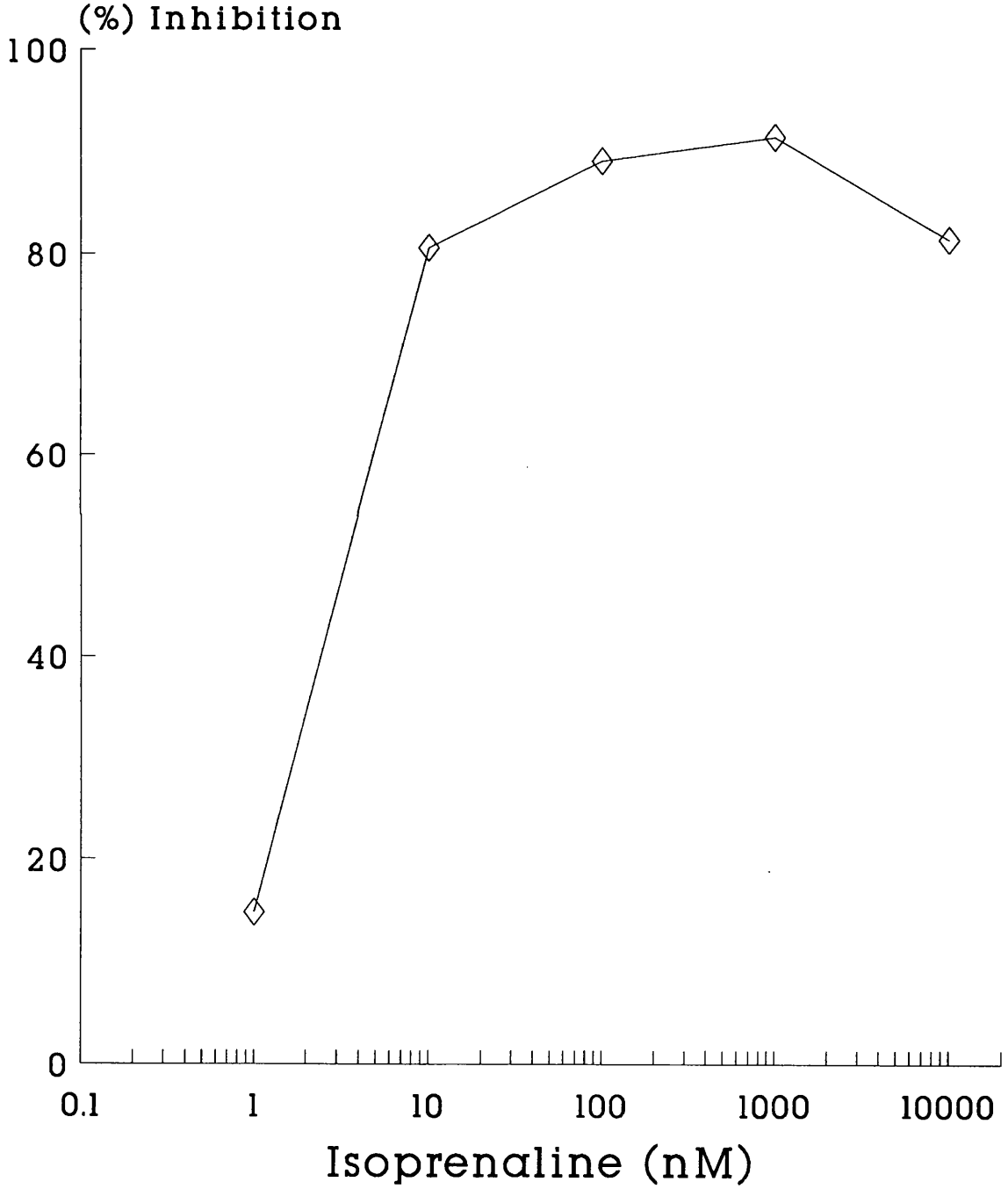


Fig 9.14 Effect of salbutamol on anti-IgE-induced histamine release from isolated human uterine myometrial mast cells. The drug was preincubated with cells for 10 min before challenge. The control release was 9.7 %, n=1.

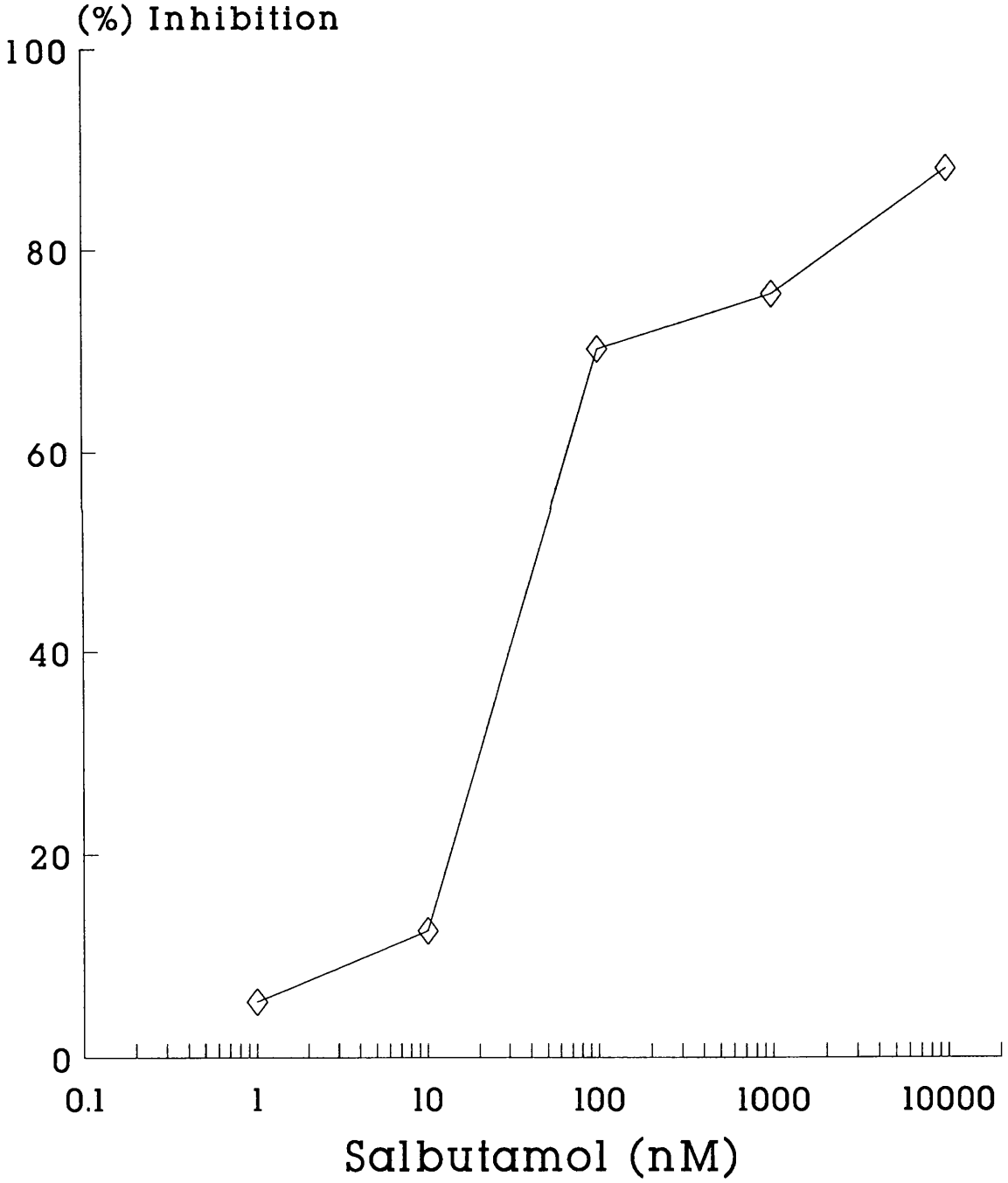


Table 9.1 Some basic properties of human mast cells isolated from the stomach mucosa (HSM) and uterine myometrium (HUM).

	HSM n=6	HUM n=4-7
Dispersion efficiency (%)	19.7±1.5	4.2±0.8
Mast cells recovered x 10^6/g tissue	0.8±0.2	0.2±0.1
Mast cells (% total)	4.8±0.8	3.1±0.7
Viable cells (% total)	93.2±2.0	83.2±3.8
Spontaneous histamine release (%)	5.7±1.3	5.0±1.2
Histamine (pg/mast cells)	2.3±0.2	1.8±0.5
Formaldehyde sensitive	87.2±1.4	84.1±6.0

Table 9.2 Histamine release from mast cells from the human stomach mucosa (HSM) and uterine myometrium (HUM) induced by compound 48/80.

Concentration (μg/ml)	Histamine release (%) HSM n=4	HUM n=3-5
10	0.8±0.7	1.5±0.3
1	-0.3±0.4	1.0±0.7
0.5	0.1±0.6	0.9±0.6
0.25	0.7±0.4	0.8±0.9
0.1	0.0±0.1	0.9±0.6

Table 9.3 Histamine release from mast cells from the human stomach mucosa (HSM) and uterine myometrium (HUM) induced by polymyxin.

Concentration (μg/ml)	Histamine release (%) HSM n=4	HUM n=3
100	2.2±0.8	2.8±1.7
33.3	1.8±0.6	1.8±0.7
10	1.7±0.5	0.6±0.3
1	0.5±0.3	0.0±0.0
0.1	0.4±0.2	0.1±0.1

Table 9.4 Histamine release from mast cells from the human stomach mucosa (HSM) and uterine myometrium (HUM) induced by substance P.

Concentration (μM)	Histamine release (%) HSM n=4	HUM n=4
50	2.8±2.5	8.7±1.1
10	0.8±0.5	3.2±1.5
5	0.8±0.5	0.7±0.4
1	0.6±0.4	0.0±0.7
0.1	0.4±0.4	-0.1±0.9

Table 9.5 Histamine release from mast cells from the human stomach mucosa (HSM) and uterine myometrium (HUM) induced by $SP_{1-4}C_{12}$.

Concentration (μM)	Histamine release (%) HSM n=4	HUM n=3
10	1.4±0.7	2.8±0.3
1	0.6±0.5	1.5±0.8
0.1	0.0±0.4	0.4±0.4
0.01	0.0±0.6	0.7±0.8
0.001	0.1±0.4	0.0±0.5

Table 9.6 Histamine release from mast cells from the human stomach mucosa (HSM) and uterine myometrium (HUM) induced by polylysine.

Concentration (μg/ml)	Histamine release (%) HSM n=4	HUM n=3
100	5.7±1.0	2.6±0.7
33.3	2.9±0.7	2.2±0.2
10	1.4±0.7	1.7±0.4
1	0.2±0.3	0.4±0.0
0.1	0.0±0.3	0.5±0.2

Table 9.7 Histamine release from mast cells from the human stomach mucosa (HSM) and uterine myometrium (HUM) induced by polyarginine.

Concentration (μg/ml)	Histamine release (%) HSM n=3	HUM n=3
100	6.4±0.8	5.1±2.0
33.3	5.4±2.1	6.4±1.3
10	7.2±4.2	5.1±2.2
1	2.0±0.4	0.9±0.5
0.1	1.8±0.4	0.1±0.1

CHAPTER TEN

GENERAL CONCLUSIONS

The present study has clearly demonstrated the existence of marked functional variations between isolated mast cells from different species, notably in their responses to a number of polybasic agents and to the inhibitory effects of anti-allergic chromones. In general, the former group of compounds which includes compound 48/80, neuropeptides and polyamino acids were most active as histamine releasers against mast cells from rat serosal cavities. Hamster peritoneal mast cells, and to a greater extent mast cells from the mouse peritoneum, were significantly less reactive. In addition, bone marrow cultured mouse mast cells (BMCMC) and enzymically dispersed mast cells from the rat intestinal mucosa, guinea pig lung and mesentery, pig lung and human lung were only very weakly responsive or essentially refractory to these agents. Human basophils in contrast, released histamine upon challenge with the polyamino acids polylysine and polyarginine, a phenomenon first described by Foreman and Lichtenstein [317]. The mechanism of action of polybasic agents in the activation of histamine release is still the subject of continuing debate. A theory was put forward recently by Repke et al [301,316] who proposed that polybasic molecules act by insertion of a hydrophobic moiety into the mast cell membrane which then permits the positively charged species to interact with a receptor or binding site which triggers the secretory process. It is then possible that the non-responsivity of certain mast cell populations to polybasic agents is due to the absence of these non-specific binding sites.

In keeping with their differential responsiveness towards various polybasic histamine liberators, the present study also extended the functional heterogeneity of isolated mast cells from different species to the inhibitory effects of anti-allergic chromones. Thus, both DSCG and nedocromil sodium were potent inhibitors of immunologically induced histamine release from rat serosal mast cells. The two drugs were, however, less active against peritoneal mast cells from the hamster and completely ineffective against these cells from the mouse. Moreover, despite their clinical utility in the treatment of bronchial asthma, the chromones were only weakly

to moderately active against dispersed human lung parenchymal mast cells, suggesting that these cells may not be the prime candidates responsible for the initial symptoms of the disease [283-285,323]. Finally immunologically induced histamine release from rat intestinal mast cells, and in addition that from the guinea pig lung and mesentery, pig lung, and human basophils was unaffected by both DSCG and nedocromil sodium. In all, these data have highlighted the difficulty in extrapolating results from one animal system to another in the screening of novel compounds that may modulate the activity of the mast cell.

The phenomenon of mast cell heterogeneity is not only confined to different species but can also be demonstrated within different tissues of a given animal. Historically, this concept was first described by Maximow [254] and later on by Enerbäck [255,324-326] on the distribution of mast cells in the gastrointestinal tract of the rat. In the present study, mast cells obtained from the enzymic dispersion of rat intestinal mucosa were sensitive to formaldehyde fixation and stained orthochromatically with alcian blue. In contrast, cells from the lung and mesentery, along with those from the peritoneum, were well preserved by both Carnoy's and formol saline and stained metachromatically with safranin. These variations in histochemistry therefore suggest the presence of distinct proteoglycans in the secretory granules. Indeed, Stevens et al [265] reported that intestinal mast cells from rats infected with the nematode Nippostrongylus brasiliensis contain the protease-resistant, less highly sulphated proteoglycan chondroitin sulphate di-B as opposed to heparin which is present in mast cells from serosal cavities [89,90,257].

In the present investigation, variations in histochemistry were also augmented by differences in functional characteristics. Isolated mast cells from the rat intestinal mucosa were, in general, much less responsive than their connective tissue counterparts from the peritoneum, mesentery and lung to the histamine releasing action of a variety of immunological and non-immunological secretagogues, as well as to the inhibitory effects of some anti-allergic compounds. Indeed, there is now a tendency by some authors to classify rat mast cells into two general groups based on the nomenclature of mucosal mast cell(s) (MMC) and connective tissue mast cell(s) (CTMC). However, the present study has also revealed functional variations between rat mast cells from

different connective tissues. Thus, isolated mast cells from the peritoneum, mesentery and lung showed a continuous gradation in reactivity towards immunological ligands, calcium ionophores, polybasic agents and histamine receptor directed compounds. In addition, mast cells from the peritoneum but not those from the lung and mesentery responded to the potentiating effect of phosphatidylserine. To further illustrate this apparent functional heterogeneity between different rat connective tissues, both DSCG and nedocromil sodium, and in addition LU 48953 and MY 1250, were potent inhibitors of immunologically induced histamine release from rat peritoneal mast cells but were only very weakly active against these cells from the mesentery and lung. These results therefore emphasise the danger in the simple classification of rat mast cells into MMC and CTMC. Clearly, mast cell heterogeneity in the rat is much more complex than this basic division.

Similarly to the situation in the rat, two types of mast cells were also detected in the human gastrointestinal tract based on their formaldehyde sensitivity; those isolated from the colonic mucosa were predominantly susceptible to formaldehyde fixation, in contrast to the colonic submucosa/muscle which contained a significantly higher percentage of formaldehyde resistant mast cells. However, despite this apparent histochemical difference, isolated mast cells from the two colonic portions were, in general, functionally similar in their responses to a variety of immunological and non-immunological stimuli. Thus, the two colonic cell preparations released histamine upon challenge with IgE-directed ligands, calcium ionophores and cytotoxic detergents but were essentially unresponsive to polybasic compounds, neuropeptides, f-met-peptide and clinical dextran. Moreover, in addition to histamine, stimulation of both colonic cell preparations with anti-IgE and the calcium ionophore A23187 resulted, in each case, in the release of the newly generated eicosanoids PGD_2 and LTC_4, with the prostanoid being the major product. In the same study, enzymically dispersed mast cells from the human lung parenchyma were compared with their colonic counterparts. These lung mast cells were largely sensitive to formaldehyde fixation, and similarly to those from the colonic mucosa and colonic submucosa/muscle, stained positively with alcian blue but negatively with safranin. Moreover, they also exhibited similar functional responses towards a variety of diverse histamine liberators.

Functional heterogeneity within human mast cells, however, was observed in their reactivities to compounds that suppressed histamine secretion. In the present study, both DSCG and nedocromil sodium produced a dose-dependent inhibition of anti-IgE-induced histamine release from human mast cells isolated from the lung parenchyma, colonic mucosa and colonic submucosa/muscle. The two drugs, in addition, exhibited tachyphylaxis against the lung cell and to a lesser extent against the colonic submucosal/muscle cell while the inhibition against the colonic mucosal cell was maintained with preincubation. Moreover, in the same investigation, this latter mast cell preparation was found to be significantly less responsive to the inhibitory effect of the β-agonists isoprenaline and salbutamol but more reactive to the H_2-antagonist ranitidine. It has been proposed [342,367] that the anti-secretory action of histamine receptor directed ligands is due to the intercalation of these compounds into the lipid bilayer in such a way as to stabilize the mast cell membrane, thereby preventing the functional changes involved in exocytosis. In this way, the differential sensitivities of human mast cells towards these compounds may indicate a difference in the structure of the cell membrane.

The role of the intestinal mast cell in inflammatory bowel disease (IBD) has been the subject of debate for many years and is still the source of dispute. Indeed, there have been many conflicting reports in the literature concerning whether these clinical disorders are accompanied by an elevation in mast cell numbers [28,386,390,391]. The present study, by using an identical enzymic dispersion procedure, has estimated that a significantly greater number of mast cells was present per gram tissue in the colonic mucosa of both Crohn's and colitic patients as compared to control subjects. Moreover, mast cells obtained from the two IBD groups contained significantly higher percentage of the formaldehyde insensitive, alcian blue positive subtype than the control group. This finding may indicate the possible recruitment of a secondary group of mast cells, probably from the submucosa or, alternatively, may point towards a change in the local tissue microenvironment which has been demonstrated to be a crucial factor in the differentiation of mast cells into their respective phenotypes [266,289-292].

In contrast to the results of Fox et al [373], isolated colonic mucosal mast cells

from patients with Crohn's disease and ulcerative colitis did not show an increase in reactivity upon challenge with anti-human IgE and concanavalin A. Although this finding failed to demonstrate an increased state of mast cell activation in IBD, it cannot rule out the potential role that such cells may play in these disorders since most of the histological features associated with IBD are best explained by the release of inflammatory and chemotactic mediators from the mast cell. With this in mind, agents that are capable in suppressing intestinal mast cell function may have therapeutic values in the management of IBD.

DSCG, widely used in the control of bronchial asthma and other allergic disorders, has also been employed in the treatment of IBD. The efficacy of the chromone in the management of these inflammatory conditions, however, has been the subject of many contrasting reports [389,399-405]. Nevertheless, a recent study by Grace et al [387] has clearly shown that, when DSCG was administered in an enema form so ensuring a high local drug concentration in the intestinal mucosa, the chromone was found to be as effective as the steroid prednisolone in the treatment of patients with ulcerative colitis. In support of this observation, the present study has demonstrated that DSCG, along with its congener nedocromil sodium, both dose-dependently inhibited anti-IgE-induced histamine release from dispersed colonic mucosal mast cells from patients with Crohn's disease and ulcerative colitis. Tachyphylaxis to the two agents, however, was observed for the former cell preparation while the inhibition seen against mast cells from colitic subjects was unaffected by preincubation; this apparent finding may also explain the lack of response of Crohn's patients towards DSCG in in vivo studies [402,405].

The mast cell, with its ability to secrete a variety of spasmogenic and chemotactic mediators including the biogenic amine histamine, has long been implicated to play an important role in reproduction [408,410,411], various uterine dysfunctions [409,417] and in the control of gastric acid secretion [421,422]. With this in mind, the present study has isolated and characterized these cells from the human uterine myometrium and stomach mucosa. Mast cells from the two locations, similarly to their lung and colonic counterparts, were predominantly sensitive to formaldehyde fixation, contained comparable amount of histamine per mast cell, released histamine in response to anti-

human IgE, concanavalin A and calcium ionophores, but were unreactive to polycationic compounds and neuropeptides. In addition, preliminary experiments have shown that mast cells from the stomach mucosa were refractory to the effect of both gastrin and acetylcholine, thereby questioning the validity of the transmission theory on the control of gastric acid secretion in man. Finally, despite their functional similarities to a variety of histamine liberators, mast cells from the uterine myometrium and stomach mucosa, like those from the lung parenchyma, colonic mucosa and colonic submucosa/muscle, exhibited subtle variations towards the anti-allergic chromones and β-agonists. However, although clearly evident, these differences are much less sharply defined than in the rat.

In total, the concept of functional, and to a lesser extent histochemical heterogeneity within mast cell populations from different species, and from different tissues within a given animal has been clearly defined in this study. The precise mechanism that account for this apparent phenomenon, however, has still to be clarified. The most plausible explanation to date concerns the regulation by 'microenvironmental' factors. Thus, mast cell precursors leave the bone marrow, circulate in the blood, and then invade target tissues where they commence their differentiation/maturation processes. The biochemical, functional and morphological characteristics of any given mast cell population is then determined by factors which are present in the local tissue microenvironment. Indeed, continuing research into how these factors, which may include agents such as the cytokines, IgE, specific antigens and even the presence of other cell types, govern the differentiation/maturation process should help to unravel further the mystery of mast cell heterogeneity.

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