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# DEMONSTRATION OF HISTAMINE $H_2$ RECEPTORS ON HUMAN MELANOMA CELLS

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Histamine induced a concentration-dependent increase in intracellular cyclic-AMP of the two human melanoma cell lines SK23 and DX3.LT5.1; maximal stimulation was obtained with 17.8μM histamine which consistently produced greater than 50-fold increases in the cyclic AMP content of both cell lines. The dose-response curve for histamine in each culture was progressively displaced to the right with increasing concentrations of the histamine $H_2$ receptor antagonist cimetidine. Ranitidine, another $H_2$ receptor antagonist also prevented the histamine-induced cyclic AMP elevation, but the $H_1$ receptor antagonists mepyramine and tripelennamine had no significant effect. These findings indicate that human melanoma cells express histamine $H_2$ receptors, stimulation of which activates adenylate cyclase with a subsequent rise in intracellular cyclic AMP. Mast cell:melanoma interactions mediated by histamine in vivo might therefore be expected to modify some aspects of melanoma cell behaviour. © 1988 Academic Press, Inc.

Since Erlich's first description of mast cells in association with neoplasms (1) numerous reports have appeared which confirm this observation for a variety of human and experimentally-induced tumours (2,3,). Despite detailed histological studies the functional significance of mast cells at the periphery of invasive tumours remains unclear. Several studies have suggested a protective role for mast cells against tumours (4,5) whereas others have emphasised an association with rapid tumour growth (3,6). The ability of the mast cell to release histamine, heparin, hydrolases, leukotrienes and other mediators suggests that mast cell:tumour cell interactions may be important in tumour cell behaviour. Since preliminary studies in this laboratory had demonstrated mast cells at the periphery of human malignant melanomas we examined the potential of two human melanoma cell lines to respond to mast cell histamine.

Three types of pharmacological histamine receptors are recognised with $H_1$ and $H_2$ receptor stimulation producing increased levels of intracellular cyclic GMP and cyclic AMP respectively. $H_1$ receptors are responsible for the histamine-induced contraction of smooth muscle from the gut or bronchus (7), effects which may be competitively inhibited by the $H_1$ receptor antagonists

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mepyramine or tripelennamine. $H_2$ receptors have been identified in several tissues (7), their stimulation leading to activation of adenylate cyclase and a subsequent rise of intracellular cyclic AMP, a response prevented by the specific $H_2$ receptor antagonists cimetidine and ranitidine (8). Histamine $H_3$ receptors have so far been identified only on cells of the brain and autonomic nervous system (9). We report here that two human melanoma cell lines express histamine $H_2$ receptors, stimulation of which results in activation of adenylate cyclase and a rapid and significant increase in intracellular cyclic AMP.

## MATERIALS AND METHODS

Dulbecco's modified Eagles medium (4.5 mg/ml glucose) was purchased from GIBCO, Paisley, Scotland. Benzyl penicillin, streptomycin and ranitidine were obtained from Glaxo Laboratories, Greenford, U.K. Foetal calf serum (heat-inactivated) and plasticware were purchased from Northumbria Biologicals Ltd., Northumberland U.K. 8-($^3$H) cAMP was purchased from Amersham International, Bucks, U.K. Pico-Fluor™ 15 scintillation cocktail was obtained from Packard Instruments Ltd., Berks, U.K. Histamine dihydrochloride, mepyramine, theophylline and 3-isobutyl-L-methylxanthine were obtained from Sigma, Poole, UK. Cimetidine was a gift from Smith, Kline and French, Welwyn Garden City, Herts, UK. All other reagents were of AnalaR grade.

### Cell Cultures

The human melanoma cell lines SK23 and DX3.LT5.1, of low and high metastatic potential in nude mice respectively, were established and subsequently obtained from the I.C.R.F., London (10). Normal human foreskin fibroblasts were donated by Dr. S.Schor (Christie Hospital, Manchester) and duct-infiltrating breast carcinoma cells were provided by Professor S. Minafra (University of Palermo, Italy).

All cells were cultured in 6-well plastic culture dishes (3.5 cm diameter) in Dulbeccos modified Eagles medium (DMEM) with 10% (v/v) foetal calf serum (FCS) supplemented with penicillin and streptomycin. The cultures were incubated at 37°C in a water-saturated atmosphere of 5% $CO_2$/95% air. When the cells were confluent the medium was removed and replaced with DMEM containing 800μM 3-isobutyl-1-methylxanthine (IBMX), a phosphodiesterase inhibitor, and the agents under investigation. After incubation at 37°C for 5 min in a water-bath the medium was removed and the cells precipitated with 6% (w/v) perchloric acid.

### Intracellular cyclic AMP assay

$HClO_4$-precipitated cells were scraped off the plastic well and homogenised in a ground glass homogeniser at 0°C. After centrifugation at 1500g for 10 mins the supernatant was removed and the protein pellet dissolved in 0.1M NaOH for Lowry protein determination (11). The supernatant was neutralised with 30% (w/v) $KHCO_3$ saturated with KCl and placed on ice for 30 mins to allow precipitation of potassium perchlorate. After centrifugation at 1500g for 10 mins the supernatant was removed, diluted with water and assayed for cyclic AMP by the competitive protein binding assay as described previously (12).

## RESULTS AND DISCUSSION

The effect of histamine on the intracellular content of cyclic AMP in human melanoma cell cultures is shown in Table 1. The melanoma cell line SK23

TABLE 1. EFFECT OF $H_1$ AND $H_2$ HISTAMINE RECEPTOR ANTAGONISTS ON THE HISTAMINE-INDUCED INCREASE OF INTRACELLULAR CYCLIC AMP IN HUMAN MELANOMA CELLS (SK23)

| | Cyclic AMP (pmol/mg cell protein) | Percent inhibition |
|---|---|---|
| DMEM | 35.7 ± 0.25 | - |
| DMEM + Histamine (17.8μM) | 3577 ± 127 | 0 |
| □ + Ranitidine(15.7μM) | 309 ± 9.3 | 91 |
| □ + Cimetidine(19.8μM) | 705 ± 20 | 80 |
| □ + Mepyramine(12.3μM) | 3293 ± 77 | 8 |
| □ + Tripelennamine(17.1μM) | 3265 ± 77 | 8 |

Monolayer cultures were grown to confluence. The culture medium was replaced with DMEM containing IBMX (800μM) as described in Methods, incubated at 37°C for 5 mins and the cells assayed for cyclic AMP. Results are mean values ± S.E.M. for triplicate determination.

responded to histamine exposure with an approximate 100-fold increase in the level of intracellular cyclic AMP. This stimulation of melanoma cell adenylate cyclase and subsequent elevation of intracellular cyclic AMP, produced after 5 min with 17.8μM histamine, was negated by the addition of approximately equimolar concentrations of histamine $H_2$ receptor antagonists. Ranitidine inhibited more than 90% of the histamine-induced rise in cyclic AMP, while cimetidine was slightly less effective with 80% inhibition. In contrast, the $H_1$ receptor antagonists mepyramine and tripelennamine, at similar concentrations to the $H_2$ antagonists, had no significant inhibitory effect on the stimulation of cyclic AMP production (Table 1). The level of histamine stimulation obtained with the SK23 cells was much greater than that recently reported for human articular chondrocytes where a 5-10 fold increase in cyclic AMP was measured following histamine treatment (13). In contrast to the pronounced effect of histamine on the human melanoma cells, similar experiments carried out in parallel with human foreskin fibroblasts or breast carcinoma cells failed to show any significant cyclic AMP response following histamine exposure (results not shown).

Confirmation of histamine $H_2$ receptor expression on human melanoma was obtained using the two cell lines SK23 and DX3.LT5.1,cells of low and high metastatic potential in nude mice, respectively. The histamine-induced rise in cyclic AMP of the two melanoma cell lines was dose-related, with significant stimulation being produced by histamine concentrations as low as 0.89μM (0.1μg/ml) for both the SK23 (Fig.1) and DX3. LT5.1 cells (Fig. 2). Maximal stimulation of cyclic AMP was observed at histamine concentrations greater than 17.8μM (2μg/ml), this being similar for both the melanoma cell lines. The cyclic AMP response curve was displaced to the right in the presence of the histamine $H_2$ receptor antagonist, cimetidine, in a concentration-dependent manner (Figs. 1 and 2). The data were subsequently used to

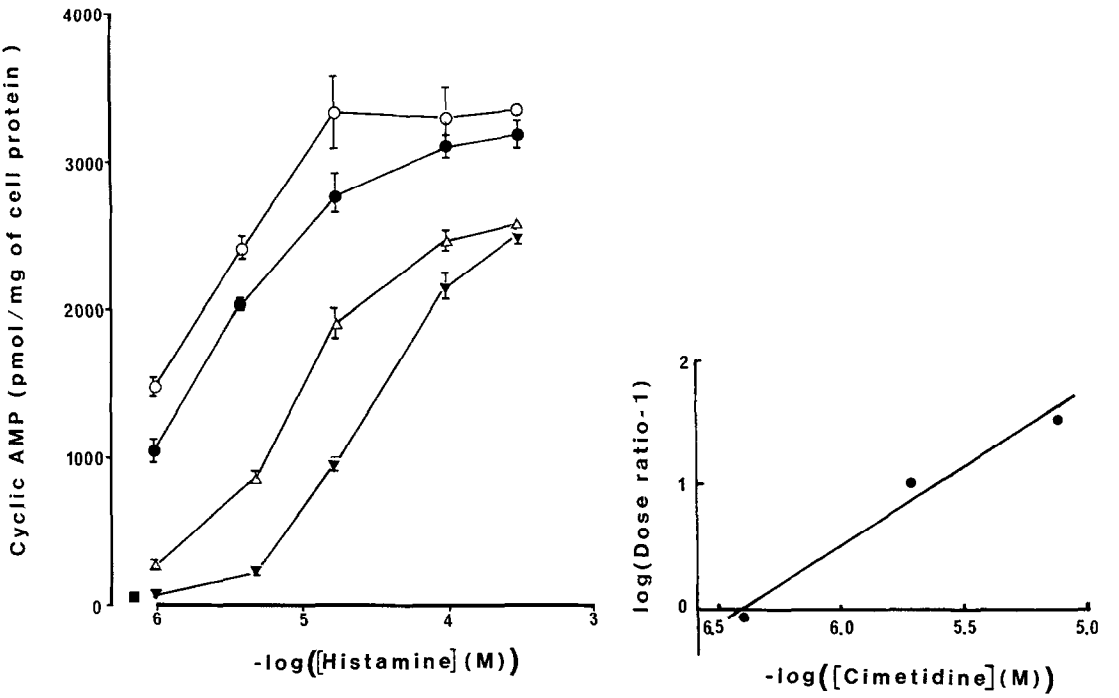


Figure 1. **EFFECT OF CIMETIDINE ON THE HISTAMINE DOSE RESPONSE CURVE OF INTRACELLULAR CYCLIC AMP IN SK23 HUMAN MELANOMA CELLS**

Confluent cell monolayers were used, the culture medium was replaced with that described in Experimental section, incubated at 37°C for 5 mins and the cells assayed for cyclic AMP, ■, control DMEM; ○, DMEM + histamine; ●, DMEM + histamine + 0.4μM cimetidine; △, + histamine + 1.9μM cimetidine; ▼, DMEM + histamine + cimetidine 7.9μM. Results are mean ± SEM (bars) for triplicate determinations.

Inset depicts the Schild plot of cimetidine concentration against the histamine-induced cyclic AMP accumulation obtained by Least squares linear regression of log (dose ratio-1) versus log (molar concentration of cimetidine) intersection of the abscissae gave $pA_2$ values of 6.4 (see text).

construct Schild plots by plotting log (dose ratio-1) against the logarithm of the molar concentration of cimetidine where the dose ratio is based on the EC50 values (i.e. histamine concentration that produces 50% of the maximum cyclic AMP response) in the absence and presence of different concentrations of cimetidine. The pA2 value (negative logarithm of the concentration required to shift the response curve by a factor of two) for the cimetidine against the histamine-induced cyclic AMP accumulation was 6.4 for both the melanoma cell lines; a value close to those reported for other $H_2$-receptor containing systems such as guinea pig ventricle (6.1) (7), and articular chondrocytes (6.3) (13). The slopes of the Schild plots shown in Figs. 1 and 2 were sufficiently close to unity (0.8 and 0.9 for the melanoma SK23 and LT5.1 respectively) to suggest that the antagonism is competitive.

The functional significance of mast cells at the periphery of specific tumours is uncertain. Mast cell degranulation in human breast carcinomas was reported to be associated with areas of infiltrative growth (14) and in rat

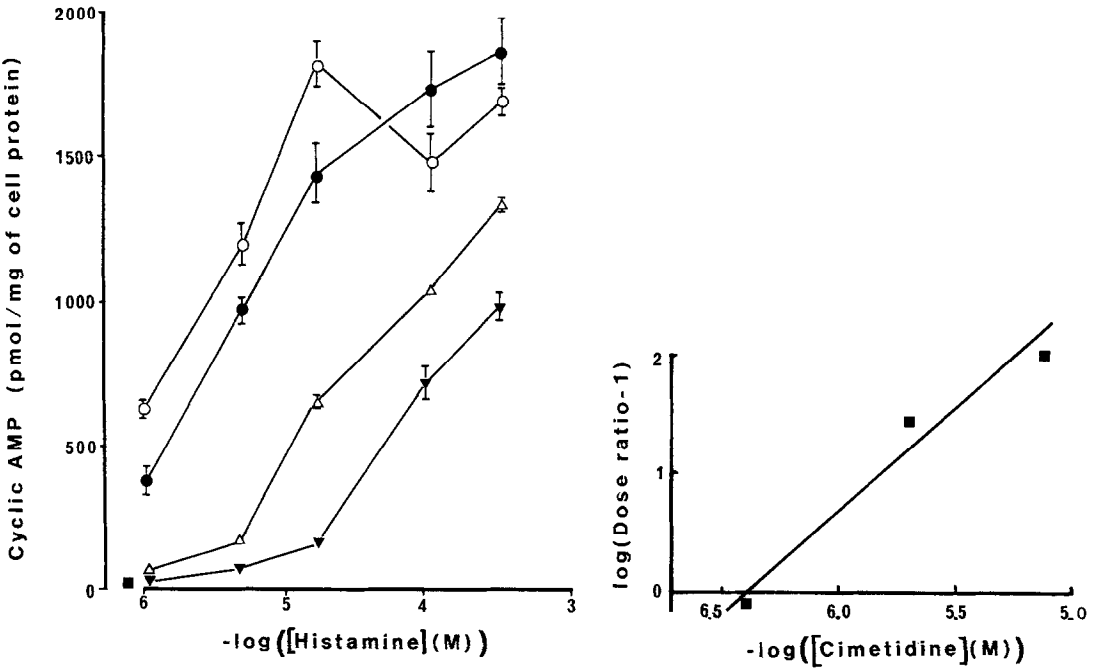


Figure 2. **EFFECT OF CIMETIDINE ON THE HISTAMINE DOSE RESPONSE CURVE OF INTRACELLULAR CYCLIC AMP IN DX3.LT5.1 MELANOMA CELLS**

The experimental conditions were identical to those described in Fig. 1. ■, control DMEM; ○, DMEM + histamine; ●, DMEM + histamine + 0.4μM cimetidine; △, DMEM + histamine + 1.9μM cimetidine; ▼, DMEM + histamine + 7.9μM cimetidine.

Inset depicts Schild plot for the DX3.LT5.1 cells and a $pA_2$ value of 6.4 (see text).

mammary adenocarcinoma degranulation was correlated with localised stromal lysis (2). Several studies have suggested a relationship between tissue histamine levels (or mast cell numbers) and tumour growth (2,15), and mast cell histamine has been reported to be mitogenic in intact tissue (15). Cloudman melanoma cells in normal mice increased their growth rate following histamine treatment, an effect possibly mediated via the stimulation of complex immunoregulatory T-cell interactions (16), but in general the direct action of histamine on specific tumour cells has received little attention.

The substantial elevation of intracellular cyclic AMP reported here for human melanoma cells following histamine exposure might reasonably be expected to change their phenotypic expression in vitro, for even in the absence of the phosphodiesterase inhibitor IBMX a significant increase in cyclic AMP was observed (result not shown). It is well known that cell proliferation is inhibited in a variety of cells when intracellular levels of cyclic AMP are raised , including Cloudman S91 melanoma (17). However, other reports have suggested that cyclic AMP may be mitogenic for some cells or tissues, such as lymphocytes (18) or liver (19). Many cellular responses to elevated cyclic AMP levels are mediated through cyclic AMP-dependent protein kinases, enzyme reactions probably involved in the control of growth , morphology and melanisation of melanoma cells (17). Our preliminary studies have shown no changes in the proliferation rate of the melanoma cells following histamine

treatment, but histamine effects on other properties such as morphology, motility, glycolysis, protein synthesis and proteinase production deserve to be examined. No demonstrable difference in the cyclic AMP response to histamine was found between the melanoma cells of high and low metastatic potential, suggesting that this response is not a phenotypic marker of metastatic potential. Indeed, the consequence of $H_2$ receptor stimulation of melanoma cells remains unclear at present, but the findings reported here indicate the potential for mast cell:melanoma interactions mediated by histamine, and further studies are in progress to elucidate the behavioural response of the melanoma cells.

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