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INHIBITION OF LYMPHOCYTE PROLIFERATION BY HISTAMINE AND RELATED COMPOUNDS NOT MEDIATED VIA H_1- OR H_2-RECEPTORS

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1 The effects of histamine and chemical analogues were examined on mitogen-stimulated human lymphocyte proliferation.

2 Compounds with selective agonist activity at either H_1- or H_2-receptors were found to inhibit proliferative responses, although N-3-methyl-histamine which does not act on either receptor was as inhibitory as histamine itself.

3 The H_2-receptor agonist, dimaprit, had a profound inhibitory effect on proliferation, however nordimaprit, which has little or no H_2-agonist activity, was more active on lymphocytes. Impromidine, although a potent H_2-agonist, failed to produce such inhibition.

4 The effects of dimaprit and nordimaprit were not reversed by H_2-receptor antagonists, cimetidine or metiamide.

5 These results do not support the view that the antiproliferative effects of histamine and related compounds are mediated via conventional H_1- or H_2-receptors.

6 SKF 93390 was found to be the most active of the dimaprit analogues tested, which could represent a novel series of potential immunosuppressive agents.

Introduction

Histamine has been shown to affect many aspects of lymphocyte functions, including proliferation (Wang & Zweiman, 1978; Beets & Dale, 1979; Brostoff, Pack & Lydyard, 1980; Ogden & Hill, 1980) and lymphokine production (Rocklin, 1976; Rocklin, Breard, Gupta, Good & Melmon, 1980), and this has led to the suggestion that endogenous histamine may act as an immunomodulator via an H_2-receptor (Busse, 1979). However, this classification has been questioned by workers who found discrepancies between H_2-receptor stimulating activity and inhibition of lymphocyte transformation (Beets & Dale, 1979; Vickers, Arlington, Jones, Martin & Melvin, 1980; Brostoff *et al.*, 1980).

In the present study we have reinvestigated the nature of the receptor involved in lymphocyte transformation by examining a group of chemical analogues of histamine. These included agonists and antagonists of H_1- and H_2-receptors and closely related analogues or 'chemical controls' which do not act on either receptor, and which were suggested by Ganellin (1980).

Methods

Mononuclear cell isolation and culture

Peripheral venous blood was obtained from healthy volunteers, not receiving any medication at the time of study, and mononuclear cells were separated from defibrinated blood by Ficoll-hypaque sedimentation as described elsewhere (Gordon & Nouri, 1981). The cell concentration was adjusted to 2×10^6 viable leukocytes/ml Dulbecco's modified Eagles medium (DMEM: Gibco) containing antibiotics (100 units penicillin/ml and 100 μg streptomycin/ml: Gibco) and supplemented with heat-inactivated (56°C, 30 min) foetal calf serum (HIFCS: Gibco). Aliquots of cell suspensions containing 2×10^5 viable leukocytes were added to 96-well (flat-bottomed type) microtitre plates. Drugs and mitogen in 20 μl volumes of DMEM were added at the beginning of culture, to give a final volume of 200 μl DMEM containing 10% HIFCS per well. The plates were incubated for 48 h, at 37°C in a humidified 5% CO_2 atmosphere. Tritiated-thymidine ([^{3}H]-TdR; 0.2 μCi, 2 Ci/mmol, Radiochemical Centre, Amersham) was added to

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each well and the plates were returned to the CO_2 incubator for a further 24 h, following which the contents of each well were collected on Whatman GF/A filter paper, by means of a multiple automated harvester. [^{3}H]-TdR incorporation (in ct/min) was measured by standard scintillation counting techniques.

Drugs

The following compounds were generously supplied by Dr C.R. Ganellin (Smith, Kline & French Laboratories Ltd): dimaprit (dimethylaminopropyl-isothiourea di HCl, SK & F 91449), nordimaprit (dimethylaminomethyl-isothiourea di HCl, SK & F 91487), SK & F 91488 (dimethylaminobutyl-isothiourea di HCl), SK & F 93390 (dimethylamino-propyl-*N,N*-dibutylisothiourea $H_2SO_4H_2O$), impromidine tri HCl (3.11×10^{-3}M in distilled water), cimetidine, metiamide, 4-pyridylethylamine HCl, 2-pyridylethylamine HCl.

Results

We have confirmed the finding (Wang & Zweiman, 1978; Beets & Dale, 1979; Brostoff *et al.*, 1980; Ogden & Hill, 1980) that histamine at high concentration (10^{-4}–10^{-3}M), partially inhibited (by up to 30%) phytohaemagglutinin (PHA, Wellcome) – stimulated [^{3}H]-TdR incorporation into human mononuclear cells, although pokeweed mitogen (PWM, Sigma) and phorbol myristic acetate (PMA, Sigma)-stimulated cells were resistant to such inhibition.

The following five findings from the data summarized in Table 1 were found to be inconsistent with the view that the inhibitory effects of histamine-related

Table 1 Effects of histamine and related compounds on lymphocyte proliferation

Compound (M)		*Class*	*% Inhibition of control [^{3}H]-thymidine incorporation* PHA (0.5 μg/ml)	PMA (0.1 μg/ml)	PWM (10 μg/ml)
Histamine	10^{-7}	H_1/H_2-	2±10	1±7	9±5
	10^{-6}	agonist	9±7	4±3	3±2
	10^{-5}		3±6	8±7	8±4
	10^{-4}		26±3*	−1±10	11±4
	10^{-3}		25±2*	3±6	6±1
3 Methyl histamine		Chemical			
	10^{-4}	control for	23±3*	19±4	12±11
	10^{-3}	histamine	27±1*	25±1*	8±2
Dimaprit	10^{-6}	H_2-agonist	−8±2	0±7	−14±5
	10^{-5}		−2±7	−8±1	9±2
	10^{-4}		10±6	−6±5	45±4**
	10^{-3}		98±0.1**	99±0.1**	98±0.1**
Nor-dimaprit	10^{-6}	Chemical	−3±6	−5±3	−1±4
	10^{-5}	control for	−18±4	−11±4	−6±0.7
	10^{-4}	dimaprit	99±0.2**	99±0.1**	99±0.8**
	10^{-3}		98±0.1**	99±0.1**	99±0.1**
2 Pyridylethylamine					
	10^{-5}	H_1-agonist	0±4	7±5	14±11
	10^{-4}		9±1	37±3**	23±8*
	10^{-3}		16±5	58±5**	62±6**
4 Pyridylethylamine		Chemical			
	10^{-5}	control for	9±2	12±2	14±11
	10^{-4}	2-pyridyl-	2±9	46±3**	23±8*
	10^{-3}	ethylamine	0±3	88±2**	64±6**
Impromidine	10^{-7}	H_2-agonist	−11±4	11±7	16±3
	10^{-6}		−2±0.5	−11±5	−2±7
	10^{-5}		14±4	−4±9	−9±12
	10^{-4}		9±8	10±4	8±5

PHA = phytohaemagglutinin; PMA = phorbol myristic acetate; PWM = pokeweed mitogen.
Values are mean ± s.e.mean of at least 10 replicate cultures from at least 2 separate donors. *$P<0.01$ and **$P<0.001$ compared with response to mitogen alone (Student's t test).

compounds on lymphocyte proliferation were mediated via H_1- or H_2-receptors.

Firstly, N-3-methylhistamine, which is inactive on conventional histamine receptors (Ganellin, 1980), was as active as histamine in inhibiting lymphocyte transformation in the same concentration range (10^{-4}–10^{-3}M).

Secondly, 2-pyridylethylamine, a selective H_1-agonist (Ganellin, 1980), inhibited the proliferative response of cells stimulated with PMA and PWM by up to 60% (10^{-4}–10^{-3}M), although this inhibition was less evident with PHA-stimulated cells. On the other hand, 4-pyridylethylamine, which is an inactive analogue of 2-pyridylethylamine on conventional H_1-receptors (Ganellin, 1980), was at least as active as 2-pyridylethylamine in inhibiting the proliferative response of cells.

Thirdly, impromidine (10^{-7}–10^{-4}M), a potent and selective H_2-agonist which is known to be more active in this respect than histamine (Ganellin, 1980), failed to reproduce the effect of histamine on lymphocytes.

Fourthly, dimaprit, another selective H_2-receptor agonist, although less active than histamine in other systems (Ganellin, 1980), and nordimaprit (SK & F 91487), its inactive homologue on conventional H_2-receptors, were both more active than histamine on lymphocyte transformation. Proliferation was inhibited at least 90% by dimaprit (3×10^{-4}M), nordimaprit (1×10^{-4}M), SK & F 91488 (3×10^{-4}M) and SK & F 93390 (3×10^{-5}M) illustrated in Figure 1.

Fifthly, Figure 2 shows that the inhibitory actions of both dimaprit and nordimaprit were not affected by the H_2-receptor antagonists, cimetidine or metiamide (10^{-4}M).

Discussion

These results strongly suggest that the inhibition of mitogen-stimulated human lymphocyte proliferation by histamine and related compounds was not mediated via a receptor comparable with those defined in other biological systems. This is less surprising in view of the high concentration required to produce a rather modest effect by histamine itself. Compounds with activity at only H_1-receptors, e.g. 2-pyridylethylamine or no activities at either H_1-receptors or H_2-receptors, e.g. N-3-methylhistamine and 4-pyridylethylamine were found to inhibit lymphocyte mitogenesis at similarly high concentrations, and were often more effective than histamine itself. The effects of the H_2-receptor agonists were of particular interest because of previous suggestions that H_2-receptors might mediate the suppressive actions on lymphocytes (Wang & Zweiman, 1978; Ogden & Hill, 1980). However, the rank order of potency in inhibiting lymphocyte proliferation was nordimaprit > dimaprit > histamine > impromidine which is the reverse of that observed for conventional H_2-receptor-mediated events. Furthermore, the H_2-receptor antagonists, cimetidine and metiamide, did not reverse the inhibitory effects of dimaprit and nordimaprit at concentrations that have been re-

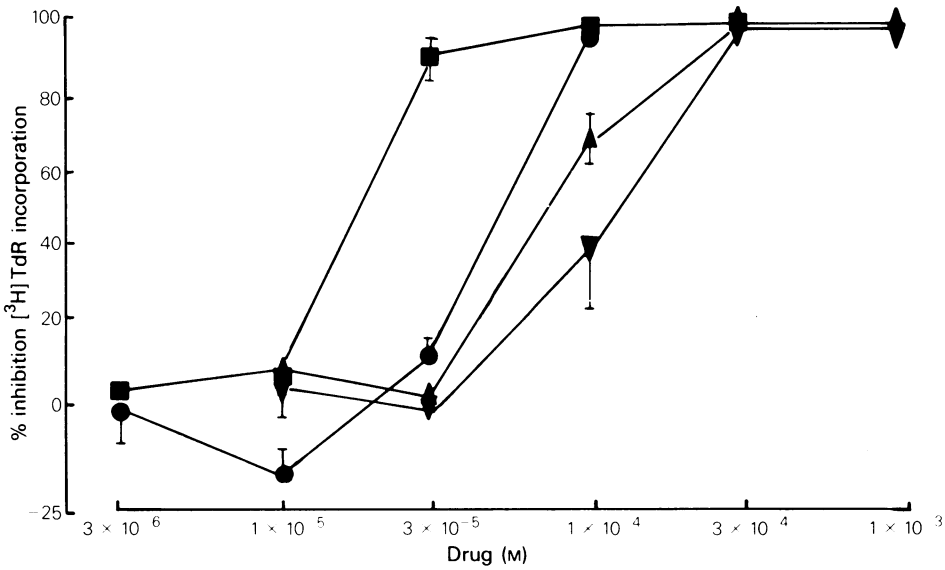

Figure 1 Percentage inhibition of [^{3}H]-thymidine ([^{3}H]-TdR) incorporation by dimaprit (▼), nordimaprit (●), SK & F 91488 (▲), and SK & F 93390 (■) of phytohaemagglutinin-stimulated (0.5 μg/ml) lymphocytes. Each value is the mean of 10–20 replicate cultures from 2–4 separate experiments; vertical lines indicate s.e.mean.

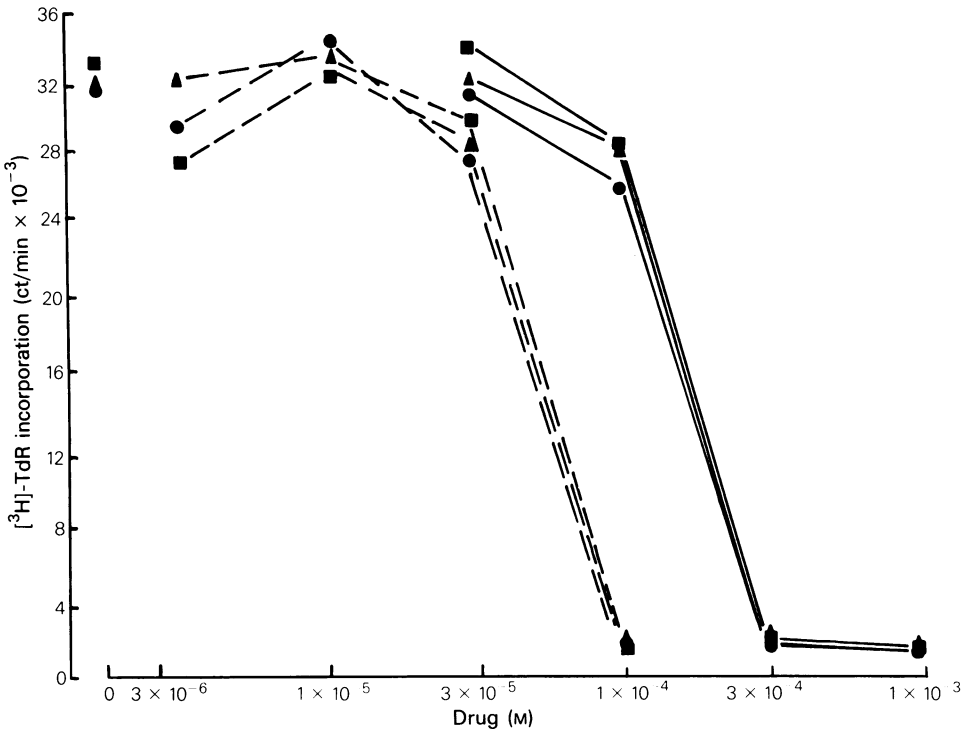

Figure 2 Inhibition by dimaprit (solid lines) and nordimaprit (broken lines) of [^{3}H]-thymidine ([^{3}H]-TdR) incorporation (ct/min) by phytohaemagglutinin-stimulated (0.5 μg/ml) lymphocytes in the absence (▲) and presence of cimetidine (10^{-4}M, ■) or metiamide (10^{-4}M, ●). Each value is the mean of 5 replicate cultures from a single representative experiment.

ported by some (Wang & Zweiman, 1978) but not by other workers (Brostoff *et al.*, 1980) to antagonize histamine itself. In the present experiments, the effect of cimetidine or metiamide on histamine-induced inhibition was equivocal (data not shown). These H_2-receptor antagonists have been found to augment mitogenesis in the absence of exogenous histamine (Beets & Dale, 1979; Gifford, Stephen, Hatfield & Schmidtke, 1980) and this effect complicates the interpretation of the reversal of inhibition by histamine-related compounds. Nevertheless, the possibility remains that human mononuclear cells bear H_2-receptors concerned with other functional activities, such as the inhibition of synthesis of complement components (Lappin & Whaley, 1980) and the generation of soluble factor(s) that suppress lymphokine production (Rocklin, 1977). These activities are expressed at lower concentrations of histamine and initial studies support the suggested involvement of H_2-receptors (Rocklin *et al.*, 1980). It remains to be seen whether the histamine-related compounds used in the present investigation will also be effective in these respects.

The profound inhibitory effects of dimaprit and nordimaprit regardless of the mitogenic stimulus, were in contrast to the limited efficacy of histamine and suggest that these compounds might represent a novel class of immunosuppressive agents. Consistent with this view was the finding that two closely related derivatives of dimaprit (SK & F 91488, SK & F 93390) included the most active so far examined.

The dimaprit derivatives possess general anti-proliferative activities (unpublished observations) and in some respects resemble anti-malarials, e.g. chloroquine, which are known to inhibit lymphocyte transformation (Hurvitz & Hirschhorn, 1965) and with which they share other common properties (inhibition of lysosomal enzyme release: Ignarro & Colombo, 1972; Vickers *et al.*, 1980; inhibition of N-methyltransferase activity: Beaven & Shaff, 1978; Thithapandha & Cohn, 1978). Their precise mechanism of action remains to be determined.

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