ACTIONS OF ADENOSINE AND GUANOSINE 3′: 5′ CYCLIC PHOSPHOROTHIOATES ON HISTAMINE SECRETION

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1. Introduction

Free peritoneal mast cells of the rat are used as a model for the study of secretion. Adenosine 3′: 5′ cyclic monophosphate itself has no effect on the secretion of histamine from mast cells, presumably because it cannot pass across the membrane into the cell. Using $N^6O^{2'}$-dibutyryl 3′: 5′ cyclic adenosine monophosphate which can penetrate into cells, and other agents which cause a rise in the intracellular level of 3′: 5′ cyclic adenosine monophosphate (cyclic AMP), it has been shown that intracellular cyclic AMP is able to exert an inhibitory effect on the secretion of histamine-containing granules from mast cells [1–4]. The inhibitory action of cyclic AMP appears to be the result of inhibition of the influx of calcium into mast cells [5,6], which is the initial and essential step in the secretory process of these cells [7].

In contrast to the action of cyclic AMP, it has been reported that 8-bromo cyclic guanosine monophosphate enhances the secretory response of mast cells in human lung [8].

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We show here that adenosine 3′: 5′ cyclic phosphorothioate has an action on histamine secretion similar to that of dibutyryl cyclic AMP, and so it follows that intracellular cyclic AMP, rather than free butyric acid which may be formed from dibutyryl cyclic AMP, inhibits the secretion of histamine. Guanosine 3′: 5′ cyclic phosphorothioate was also found to inhibit histamine secretion.

2. Methods

The source and preparation of rat peritoneal mast cells has been described [9]. Aliquots of cells were incubated in a medium based on Tyrode solution in which bicarbonate–CO_2 buffer was replaced by Hepes [9]. Histamine was assayed fluorimetrically [10] and for each aliquot of cells, both the histamine released into the incubating medium and that remaining in the cell pellet was determined so that for each sample the percentage of histamine released from the cells could be calculated as shown:

$$\frac{\text{Amount of histamine in supernatant}}{\text{Amount in supernatant + amount in pellet}} \times 100\%$$

$N^6O^{2'}$-dibutyryl 3′: 5′ cyclic adenosine monophosphate (db cAMP) was obtained from Sigma Chemical

Co., London. Adenosine and guanosine 3′: 5′ cyclic phosphorothioates were synthesized as in [11]. The ionophore A23187 (Eli Lily) was first dissolved in ethanol and then diluted into physiological saline before addition to the cells: the final concentration of ethanol being too small to affect the response of the cells to antigen stimulation.

3. Results and discussion

Adenosine 3′: 5′ cyclic phosphorothioate produced a concentration-dependent inhibition of histamine secretion induced by antigen stimulation. A comparison of the activity of $N^6O^{2'}$-dibutyryl 3′: 5′ cyclic adenosine monophosphate with that of adenosine 3′: 5′ cyclic phosphorothioate was made using a 2+2 biological assay design. Cells were preincubated with the compounds for 10 min before antigen stimulation since preliminary experiments showed that there was no increase in the inhibitory effect with preincubation over the period 10–30 min. The results depicted in fig.1 are the means from 3 experiments in which 2 concentrations of each compound were used to inhibit histamine secretion. In each experiment, a particular concentration was tested on quadruplicate samples of cells. No difference in activity between dibutyryl cyclic AMP and adenosine cyclic phosphorothioate was observed. An analysis of variance was carried out on the results of these experiments and it was determined that differences between the 2 compounds as large or larger than those observed would be common on a chance basis ($P > 0.05$). The analysis of variance

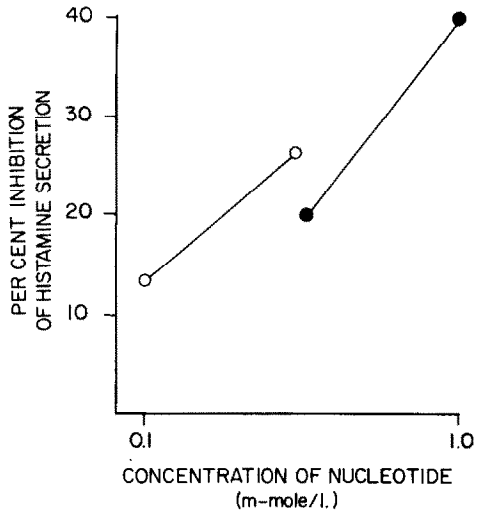

Fig.1. Concentration–response relationships for the inhibition of antigen-induced histamine secretion by $N^6O^{2'}$-dibutyryl 3′: 5′ cyclic adenosine monophosphate (○——○) and adenosine 3′: 5′ cyclic phosphorothioate (●——●). Each point is the mean of quadruplicate determinations in each of 3 separate experiments. Histamine secretion in the absence of inhibitor ranged from 7–33% total.

Table 1
Inhibition by dibutyryl cyclic AMP (db cAMP) and adenosine cyclic phosphorothioate (cAMPS) of histamine secretion induced by antigen (Ag) or A23187 (0.6 μmol/l)

		Inhibition (%)							
		Exp. a		Exp. b		Exp. c		Mean	
Release by		Ag	A23187	Ag	A23187	Ag	A23187	Ag	A23187
db cAMP	0.3	–	–	28	8	42	5	35	6
mmol/l	1.0	71	15	62	18	–	–	66	16
cAMPS	0.3	–	–	24	5	21	0	23	3
mmol/l	1.0	60	18	–	–	38	0	49	9
Control secretion	(%)	37	81	44	77	58	42		

Inhibition is expressed as % histamine release in the absence of nucleotide (control). Each value is a mean of duplicate determinations

provided no evidence against the hypothesis that the concentration–response lines are parallel, and it rejected the null hypothesis that the gradient of the concentration–response lines is zero.

It has previously been reported that dibutyryl cyclic AMP inhibits antigen-induced histamine release but has no effect on histamine release induced by the ionophore A23187 [5]. Table 1 shows that both dibutyryl cyclic AMP and adenosine cyclic phosphorothioate produce a marked, dose-related inhibition of antigen-stimulated histamine secretion whilst having little or no effect on the secretion induced by the calcium inophore A23187. Again, cells were preincubated for 10 min with the nucleotide before addition of the histamine releasing stimulus.

Table 2 demonstrates that guanosine 3′: 5′ cyclic phosphorothioate produces a concentration-dependent inhibition of antigen stimulated histamine secretion. Cells were preincubated with the nucleotide for 10 min before stimulation. Concentrations down to 0.1 μmol/l were tested but only those in the range 0.01–1.0 mmol/l produce an effect on histamine secretion.

The nucleoside cyclic phosphorothioates were synthesized to obtain analogues of such cyclic phosphates which would not be hydrolysed readily by phosphodiesterase and which might be able to enter the cell. Although the dibutyryl derivatives of the cyclic nucleotides have these properties, alternative analogues were thought to be desirable because of the reported side-effects of butyric acid [12–14]. The adenosine cyclic phosphorothioate has been shown to activate protein kinase, to be hydrolysed by phosphodiesterase although considerably more slowly than cyclic AMP [11] and to mediate cell aggregation in *Dictyostelium discoideum* [15]. It also mimics the action of dibutyryl cyclic AMP on amylase secretion from rat parotid slices [16]. This is probably an indication of sufficient lipid solubility of this compound to allow its entry into cells. We show here that in the process of histamine secretion from rat mast cells, adenosine 3′: 5′ cyclic phosphorothioate has an activity which is indistinguishable from that of $N^6O^{2'}$-dibutyryl 3′: 5′ cyclic adenosine monophosphate. The inhibition of secretion produced by these agents is thought to result from an increase in intracellular 3′: 5′ cyclic adenosine monophosphate level.

Table 2
Inhibition by guanosine 3′: 5′ cyclic phosphorothioate of antigen-induced histamine secretion

Nucleotide (mmol/l)	Experiment		
	a	b	c
0.01	–	–	12
0.1	26	54	27
1.0	40	–	–
Control secretion (%)	72	12	36

Inhibition is expressed as % secretion in the absence of nucleotide (control). Each value is a mean of duplicate determinations

Adenosine cyclic phosphorothioate inhibits histamine secretion induced by antigen and has little if any inhibitory effect on secretion induced by the calcium ionophore A23187. A similar action has already been reported for dibutyryl cyclic AMP [5] and since the ionophore and antigen are thought to initiate release by increasing membrane permeability to calcium and allowing calcium to enter the mast cell [6,7], it follows that adenosine cyclic phosphorothioate and dibutyryl cyclic adenosine monophosphate selectively inhibit antigen-induced histamine release by interfering with calcium transport into mast cells mediated by the antigen stimulus. It has been shown that dibutyryl adenosine cyclic monophosphate inhibits antigen-induced calcium-45 uptake but not A23187-induced calcium-45 uptake in mast cells [6].

Guanosine cyclic phosphorothioate inhibits histamine secretion induced by antigen whereas it has been found that 8-bromo cyclic guanosine monophosphate enhanced histamine release induced by antigen from human lung mast cells [8]. 8-Bromo and dibutyryl cyclic guanosine monophosphate are both without effect on rat mast cells at concentrations up to 1 mmole/l (J. C. F., unpublished data). The basis for these differences, and the mechanism of action of guanosine cyclic phosphorothioate in inhibiting histamine secretion remains to be determined.

The nucleoside cyclic phosphorothioates are valuable new probes of cyclic nucleotide effects and should help to assess the role of cyclic nucleotides in secretion and other biochemical processes.

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