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The actions of inhibitors of diacylglycerol kinase and diacylglycerol lipase on histamine release from rat peritoneal mast cells

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1 RHC 80267 and R59 022 are selective inhibitors of diacylglycerol (DAG) lipase and DAG kinase enzymes respectively. These inhibitors were examined with regard to their effects on oleoylacetylglycerol (OAG)-and anti-IgE- induced histamine secretion in rat peritoneal mast cells.

2 RHC 80267, 10 μM and R59 022, 50 μM both enhanced OAG-induced histamine release by 30% and 40% respectively.

3 In the concentration range 3–30 μM, R59 022 enhanced anti-IgE-induced histamine release by up to about 40%, whereas RHC 80267 was without effect.

4 The enhancement of anti-IgE-induced histamine release by R59 022 is consistent with a role for protein kinase C in transducing immunological signals to rat peritoneal mast cells.

5 The lack of effect of RHC 80267 in this situation may indicate that in the mast cell, DAG kinase is more active than DAG lipase in degrading physiological levels of DAG.

Introduction

Protein kinase C is regarded as having a role in the transduction of many signals to the cell surface into biological responses (Nishizuka, 1984). Protein kinase C, a calcium and phospholipid-dependent enzyme is activated by endogenous diacylglycerol (DAG) produced during phosphoinositol breakdown (Kawahara *et al.*, 1980). The effects of DAG in the cell are transient as DAG can be rapidly metabolized by either DAG kinase or DAG lipase enzymes (Nishizuka, 1986). DAG kinase converts DAG to phosphatidic acid, whereas DAG lipase catabolizes DAG to monoacylglycerol and a fatty acid, commonly arachidonic acid. The phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) has a DAG-like structure and will directly activate kinase C (Castagna *et al.*, 1982) but it is not subject to enzymatic degradation (Blumberg, 1980). Consequently, TPA produces a long lasting action which is useful in probing the consequences of protein kinase C activation.

Activation of protein kinase C can, under certain conditions, increase mast cell activation induced by a ligand-receptor interaction at the membrane. For example in rat peritoneal mast cells we have shown that at low concentrations (10–30 ng ml^{-1}) and short periods of preincubation ($\leq$ 10 min) TPA can interact synergistically with anti-IgE and A23187 such that histamine secretion induced by anti-IgE or A23187 plus TPA is greater than the sum of the histamine secreted in response to TPA and either anti-IgE or A23187 added separately (Katakami *et al.*, 1984; Heiman & Crews, 1985; Cantwell & Foreman, 1986). Also, TPA produces histamine secretion in the absence of a receptor-ligand interaction at the cell membrane but in this case the secretion is slow, taking 90 min to reach maximum (Cantwell & Foreman, 1987). In contrast, higher concentrations of TPA ($>$ 100 ng ml^{-1}) and longer periods of preincubation ($>$ 10 min) produce inhibition of immunologically-mediated histamine release (Sagi-Eisenberg & Pecht, 1984; Cantwell & Foreman, 1986).

The aim of this paper was to investigate the effect of inhibiting the breakdown of exogenous and endogenous diacylglycerols on histamine release. 6-[2-[4-[(4-fluorophenyl)phenylmethylene]-1-piperidinyl] ethyl] -7-methyl-5H-thiazolo[3,2-α]pyrimidin-5-one (R59 022) was used to inhibit diacylglycerol kinase. It has previously been shown to have an IC_{50} of about 4 μM in intact platelets (De Chaffoy De Courcelles *et al.*, 1985). 1,6-di (O-(carbamoyl)cyclohexanone oxime) hexane (RHC 80267) was used to inhibit diacylglycerol lipase. It has an IC_{50} for platelet diglyceride lipase of about 4 μM (Sutherland & Amin, 1982).

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Methods

Male Sprague-Dawley rats (250–350 g) were used for these experiments. Mast cells were obtained from the peritoneal cavity as previously described (Cantwell & Foreman, 1986). In all experiments 0.8 ml aliquots of cells were preincubated for 5 min at 37°C to permit temperature equilibration and then 0.1 ml of a solution of RHC 80267, R59 022 or Tyrode solution was added. Incubation proceeded for 15 min at 37°C which has been shown to be adequate for the maximum expression of the effect of R59 022 (De Chaffey De Courcelles *et al.*, 1985). The same preincubation time was used for RHC 80267 since there appears to be no data on the time-course of its effect in whole cells. The cells were then stimulated by adding either oleoylacetylglycerol (OAG) or anti-IgE in a volume of 0.1 ml. A further incubation of 10 min was allowed for histamine release to occur at 37°C. The release reaction was terminated and histamine release determined and calculated as previously described (Cantwell & Foreman, 1986). Histamine release occurring in the absence of any drug or releasing agent (spontaneous release) was less than 7% of total and has been subtracted from the stimulated release.

The composition of Tyrode solution was (mM): NaCl 134, KCl 2.7, glucose 5.6, NaH_2PO_4 0.4, HEPES (4-(2-hydroxyethyl)-1-piperazine ethane sulphonic acid) 20 and Ca^{2+} 1.0.

Goat anti(rat IgE) serum was obtained from Miles Laboratories Ltd. OAG was obtained from Sigma and was prepared as a stock solution of 75.28 mM in dimethyl sulphoxide (DMSO). RHC 80267 was a gift from Revlon Health Care Group, U.S.A. and was prepared as a stock solution of 20 mM in DMSO. R59 022 was supplied by Janssen Life Science Products, Belgium and it was dissolved in DMSO at a concentration of 20 mM. Further dilutions of drugs dissolved in DMSO were made in Tyrode solution. DMSO did not affect spontaneous histamine release or anti-IgE-induced histamine release at the levels associated with final concentrations of R59 022 or RHC 80267 used. However, at R59 022 concentrations greater than 50 μM the DMSO vehicle began to exert some inhibitory action on anti-IgE-induced histamine release. RHC 80267 and R59 022 did not interfere with the histamine assay.

Results

The DAG kinase inhibitor R59 022 50 μM and the DAG lipase inhibitor RHC 80267 10 μM enhanced OAG-induced histamine release (Figure 1). The inhibitors themselves induced small histamine releases: 0.9 ± 0.4% ($n = 13$) for RHC 80267 and 0.3 ± 0.1% ($n = 9$) for R59 022. After subtraction of inhibitor-induced release from the combined OAG plus inhibitor response, RHC 80267 was found to increase histamine release from 14.6% ± 4.4% to 21.7% ± 3.5%; R59 022 increased release from 14.6 ± 4.4% to 16.8% ± 4.6%. The increase produced by RHC 80267 was, by paired t test, statistically significant ($0.05 > P > 0.02$) but the increase produced by R59 022 was not significant ($P > 0.05$).

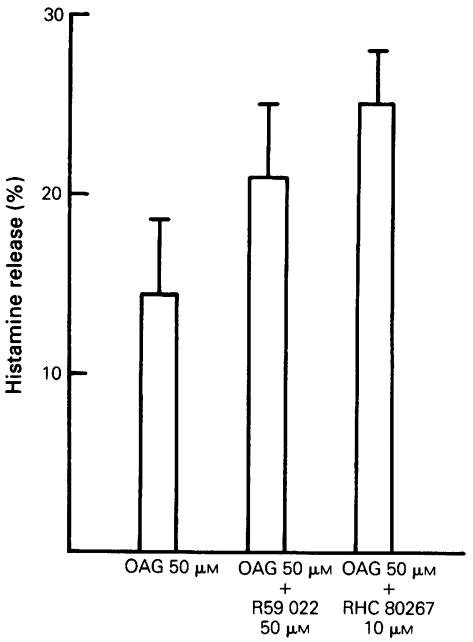

Figure 1 The effect of R59 022 50 μM and RHC 80267 10 μM on oleoylacetylglycerol (OAG)-induced histamine secretion. Cells were preincubated at 37°C for 15 min with either Tyrode solution as a control or one of the inhibitors and then stimulated with OAG (50 μM). Histamine release was allowed to proceed for a further 10 min. Histamine releases by R59 022 and RHC 80267 alone were 4.2 ± 2.5% and 3.5 ± 1.3% respectively. The results indicate the means of 3 experiments; s.e.mean shown by vertical lines. Histamine release by RHC 80267 plus OAG was significantly greater than release by OAG alone (paired t test: $0.05 > P > 0.02$).

The effect of the DAG lipase inhibitor RHC 80267 on the time-course of histamine release by OAG is illustrated in Figure 2. RHC 80267 10 μM had the following effects on the time-course; (i) it shifted the time-response curve to the left; (ii) it increased the maximum level of histamine release from approximately 18% to 24%; (iii) it extended the incubation time at which the maximum response occurred from 20 min to 30 min.

Figure 3 shows the effect of RHC 80267 and R59 022 on histamine release induced by the immunological stimulus, anti-IgE. At the dilution of 1:400,

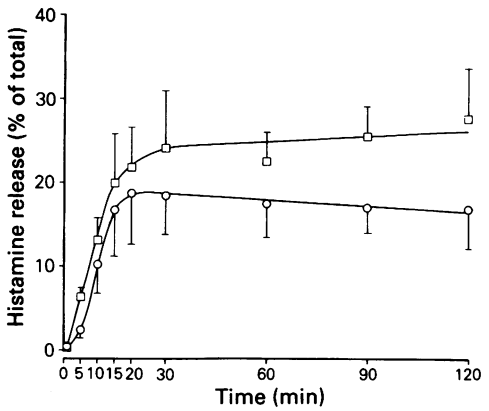

Figure 2 The effect of RHC 80267 on the time-course of oleoylacetylglycerol (OAG)-induced histamine release. Cells were incubated with OAG at 37°C for the times shown on the abscissa scale, (○) histamine release by OAG 50 μM; (□) histamine release by OAG following 15 min preincubation at 37°C with RHC 80267 10 μM. RHC 80267 alone released 3.5 ± 1.2% of cell histamine: this release has been subtracted from the combined response to OAG plus RHC 80267. The results represent the means of 3 separate experiments; s.e.mean shown by vertical lines.

histamine release by anti-IgE was near to maximum. The DAG kinase inhibitor R59 022 50 μM increased the level of histamine released at all anti-IgE concentrations tested. In contrast, RHC 80267 10 μM did not increase the histamine release at any of the anti-IgE concentrations tested (Figure 3).

Figure 4 shows the dose-response relationship for the enhancement of anti-IgE-induced histamine release by the DAG kinase inhibitor R59 022. A concentration-related effect of the compound R59 022 was observed between 3 and 30 μM and because of interference by the vehicle DMSO, higher concentrations were not tested. Over the concentration range 3 to 30 μM, the DAG lipase inhibitor RHC 80267 did not change the response to anti-IgE.

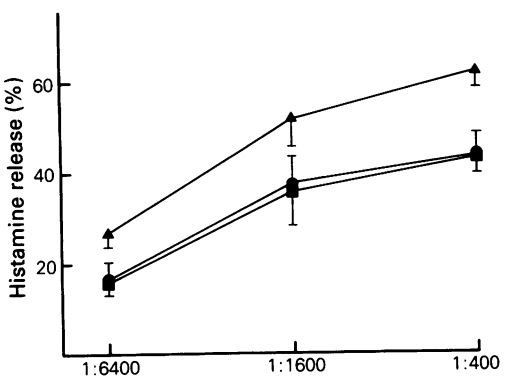

Figure 3 The effect of R59 022 and RHC 80267 on anti-IgE-induced histamine secretion. Cells were preincubated at 37°C for 15 min with Tyrode solution as a control or with one of the inhibitors, and were then challenged with anti-IgE. Histamine release was allowed to continue for 10 min; (●) histamine release by anti-IgE alone; (■) histamine release by RHC 80267 10 μM plus anti-IgE; (▲) histamine release by R59 022 50 μM plus anti-IgE. Histamine releases by R59 022 and RHC 80267 alone were 4.2 ± 2.5% and 3.5 ± 1.3% respectively and these releases have been subtracted from the combined responses of drug plus anti-IgE. The results represent the means of 3 experiments; s.e.mean shown by vertical lines.

Discussion

There is evidence that the calcium- and phospholipid-dependent protein kinase, kinase C is involved in the transduction of ligand-receptor interaction on the mast cell membrane to the secretory response of these cells. When TPA was used to activate kinase C, first evidence indicated that calcium as an intracellular second messenger interacted synergistically with activated kinase C to initiate histamine secretion (Katakami *et al.*, 1984). Partially purified kinase C has been isolated from the histamine-containing rat basophilic leukaemia cells (Sagi-Eisenberg & Pecht, 1984). Furthermore, kinase C-mediated protein phosphorylation has been demonstrated in rat peritoneal

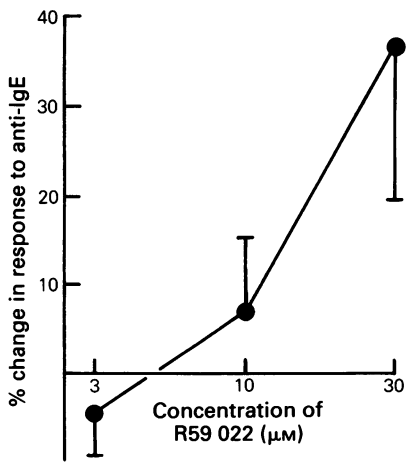

Figure 4 Concentration-response relationship for the enhancement of anti-IgE-induced histamine release by the diacylglycerol (DAG) kinase inhibitor R59 022. Enhancement is shown as a percentage change induced by R59 022 of the histamine release induced by anti-IgE (1:400 dilution) in the absence of the inhibitor. The anti-IgE-induced histamine release in the absence of R59 022 was 35 ± 10% of total. Results are the means from three experiments; s.e.mean shown by vertical lines.

mast cells, (Heiman & Crews 1985; Sagi-Eisenberg *et al.*, 1987).

The hypothesis that free cytosolic calcium and activated kinase C act in consort as second messengers may, however, be too simplistic. Firstly, in rat peritoneal mast cells it has been shown that TPA, at higher concentrations or at longer preincubation times, inhibits histamine secretion induced by antigen and anti-IgE (Cantwell & Foreman, 1986). Moreover, we found no correlation between secretory response and TPA-induced protein phosphorylation (Sagi-Eisenberg *et al.*, 1987). In fact, it has been shown that protein kinase C activation, whilst it can act in consort with calcium as a second messenger, also serves to reduce ligand-receptor-dependent calcium signals in histamine-containing cells (Sagi-Eisenberg *et al.*, 1984).

OAG induces histamine release from rat mast cells, as does TPA (Cantwell & Foreman, 1987) presumably by substituting for endogenously produced DAG. Endogenous DAG is rapidly metabolized in the cell (Nishizuka, 1986) by kinase and lipase enzymes. In this paper we have shown that inhibitors of both DAG metabolizing enzymes increase the histamine release induced by OAG. As OAG is metabolized like endogenous DAG, inhibition of its metabolism would therefore be expected to increase its effect. We assume, of course, that the inhibitors RHC 80267 and R59 022 are acting specifically on DAG metabolizing enzymes. There is some evidence that these drugs are selective in their actions. RHC 80267 has an IC_{50} for DAG lipase of about 4 μM whereas it produces only slight inhibition of phospholipases A_2 and C at concentrations of 100–300 μM (Sutherland & Amin, 1982). Similarly, R59 022 with an IC_{50} for DAG kinase of about 4 μM had no effect on phosphoinositide phosphodiesterase, phosphatidyl inositol kinase and phosphatidyl inositol 4-phosphate kinase (De Chaffoy de Courcelles *et al.*, 1985). RHC 80267 increased OAG-induced histamine release at a concentration of 10 μM whereas R59 022 failed significantly to have an effect at a concentration of 50 μM; well above its IC_{50} value for the isolated enzyme. In contrast, R59 022 produced a dose-dependent increase of anti-IgE-induced histamine release from 3 to 30 μM while RHC 80267 at the concentration which increased OAG-induced release (10 μM) was without effect on anti-IgE-induced release. It is interesting that RHC 80267 produces a greater effect than R59 022 on the OAG-induced release of histamine, possibly indicating that the lipase enzyme is responsible for metabolizing the greater part of exogenous diglyceride.

The observation that the DAG kinase inhibitor R59 022 enhances immunologically mediated histamine release suggests that the triggering events between the IgE-anti-IgE interaction at the mast cell membrane and the secretory process involve activation of protein kinase C. Hence, endogenous DAG produced by the IgE-directed ligand (Beaven *et al.*, 1984) has its metabolism reduced in the presence of R59 022 and thus the increased activation of protein kinase C results in enhanced histamine release. It is noteworthy that the DAG-lipase inhibitor RHC 80267 is without effect on the secretion induced by the IgE-directed ligand in contrast to its effect on OAG-induced release. We suggest, therefore, that for reasons of accessibility or compartmentalization, DAG lipase may be involved in the metabolism of exogenous DAG whereas the kinase may be more important for the inactivation of the endogenous DAG signal. However, the observed difference between the effects of the DAG-lipase and DAG-kinase inhibitors on OAG- and anti-IgE-induced histamine release may result from different enzyme concentrations within the cell or differences between the turnover numbers or affinities of the two enzymes. Furthermore, a combination of these mechanisms might be used to explain the data.

It is noteworthy that in neutrophil leukocytes both RHC 80267 and R59 022 potentiate OAG-induced superoxide generation whereas only R59 022 potentiated superoxide generation by C3b or fMet-Leu-Phe receptor activation (Muid *et al.*, 1987). This is exactly comparable to our findings in the rat mast cell.

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