

Modulation of human leukocyte histamine release by sn-1,2-isopropylidene-3-decanoyl-glycerol and decanoic acid cyclopentyl methylester in comparison with effects of synthetic diacylglycerols and a phorbol ester

H. BERGSTRAND,¹ B. LUNDQUIST¹ & P. MICHELSEN²

¹Pharmacological Laboratory, Research and Development Department, AB Draco*, Lund, and ²Dept. of Organic Chemistry 1, University of Lund, Sweden

Previous studies have shown that the glyceride derivative, sn-1,2-isopropylidene-3-decanoyl-glycerol (IpOCOC₉), can trigger human leukocyte histamine release. Approximately 25 % of the total cellular histamine content is extruded in the presence of 206 μ M of IpOCOC₉; at 69 μ M, however, the secretagogue action of the compound is marginal. The characteristics of the release induced by IpOCOC₉ are closely similar to those reportedly recorded at hyperosmolar triggering of basophils with mannitol, and in many respects they also mimic those observed at phorbol ester-induced histamine release. The compound decanoic acid cyclopentyl methylester (DACPME), a structural analogue of IpOCOC₉, fails to induce histamine release. IpOCOC₉, but not DACPME, stimulates human polymorphonuclear leukocyte cytosolic Ca²⁺- and phospholipid-dependent histone III-S kinase activity (unpublished observations). The secretagogue action of IpOCOC₉ has therefore tentatively, at least partly, been attributed to a direct protein kinase C activation. In the present studies, we examined the influence of IpOCOC₉ and DACPME on histamine release triggered by an ensuing exposure to anti-IgE, the calcium ionophore A23187, formyl-methionyl-leucyl-phenylalanine (FMLP), or 4 β -phorbol 12-myristate 13-acetate (PMA). It is shown that IpOCOC₉-treatment of cells results in either enhancement or reduction of the release induced by anti-IgE or by A23187, whereas FMLP-induced release is consistently reduced and PMA-induced release consistently enhanced by such a treatment. Treatment of cells with DACPME enhances but does not reduce anti-IgE-triggered release, whereas FMLP-induced release is not affected. Pretreatment of the cells with other putative protein kinase C activators like PMA, sn-1-oleoyl-2-acetyl-glycerol (OAG), 1,2-dioctanoyl-glycerol (DiC₈) or the glycerol derivative sn-1,2-diacetyl-3-decanoyl-glycerol (DiC₂OCOC₉) affects secretagogue-induced basophil histamine release according to specific patterns similar to but not identical with those recorded for IpOCOC₉ and DACPME. Thus, e.g., DiC₂OCOC₉ consistently reduces but does not enhance anti-IgE-triggered release. These data show that limited structural changes of IpOCOC₉ may qualitatively affect its modulating properties in the human basophil histamine release system.

Key words: anti-IgE; A23187; basophil; DiC₈; IpOCOC₉; FMLP; OAG.

Accepted for publication 24 May 1988

Protein kinase C is the only hitherto identified cellular receptor for the potent inflammation-inducing and tumor-promoting phorbol esters.

The phorbol esters are thought to mimic the action on protein kinase C of diacylglycerols, second messengers which at various forms of cell activation are generated by phospholipase C-catalyzed degradation of polyphosphoino-

*Subsidiary of AB Astra, Södertälje, Sweden

sitides (2, 5, 26, 34). In the human leukocyte histamine release system, the difficulty of obtaining reasonable amounts of sufficiently pure basophils has hampered direct biochemical studies on this pathway for signal transduction at triggering of the cells for mediator release with various secretagogues. However, the observation that 4 β -phorbol 12-myristate 13-acetate (PMA) induces histamine release from these cells (41, 42) is an indication that a protein kinase C activity may constitute a link in one signal transduction pathway in the basophil. When studying effects of glycerol derivatives with the aim of exploring this possibility further, we observed that the compound sn-1,2-isopropylidene-3-decanoyl-glycerol (IpOCOC₉) triggered human leukocyte histamine release apparently by a non-cytolytic process (9). At 206 μ M IpOCOC₉ approximately 25 % of the total cellular content was secreted, whereas the release recorded at 69 μ M was marginal. The general characteristics of this release process were found to correspond closely to those previously reported for release triggered by hyperosmolar mannitol (9, 18), and also in many respects to mimic those recorded at triggering of cells by PMA. The structural analogue of IpOCOC₉, decanoic acid cyclopentyl methylester (DACPME), did not trigger basophil histamine release (9). IpOCOC₉, but not DACPME, was found to stimulate the Ca²⁺- and phospholipid-dependent histone III-S kinase activity of a human polymorphonuclear leukocyte cytosol (H. Olsson & H. Bergstrand, manuscript in preparation). A direct protein kinase C activation, therefore, possibly contributes to the secretagogue activity of IpOCOC₉. An examination of the influence on IpOCOC₉-induced release of a number of agents which are thought to interact with Ca²⁺- and phospholipid-dependent protein kinase (PKC) activities further characterized the secretagogue properties of IpOCOC₉ in relation to those of PMA. The effects on IpOCOC₉-induced histamine release of various putative inhibitors of protein kinase C did not closely conform to their influence on PMA-induced release (8). The reason for this is not clarified; one working hypothesis includes the possibility that PMA

and IpOCOC₉ act principally on different entities of the protein kinase C family.

In the present report, we examined the *modulatory* effects of IpOCOC₉ and DACPME on histamine release induced by the IgE-receptor dependent stimulus anti-IgE, the calcium ionophore A23187, formyl-methionyl-leucyl-phenylalanine (FMLP) and PMA. Pretreatment of cells was performed with nontriggering as well as triggering concentrations of IpOCOC₉. Pretreatment of cells was also performed with a closely related glyceride derivative, sn-1,2-diacetyl-3-decanoyl-glycerol (DiC₂OCOC₉), with the synthetic diacylglycerols dioctanoyl-glycerol (DiC₈) and sn-1-oleoyl-2-acetyl-glycerol (OAG), and with PMA. The results of these studies form the basis of the present report.

MATERIAL AND METHODS

Materials were obtained or prepared as described elsewhere (8, 9). The biologically inactive 4 α -phorbol 12-myristate 13-acetate (4 α -PMA) was obtained from LC Services Corporation, Woburn, USA. The compounds DiC₂OCOC₉, DiC₈ and OAG were synthesized as described below. OAG was prepared from commercial 2,3-o-isopropylidene-sn-glycerol (Sigma), optical purity approximately 90 %, according to Buchnea (11). OAG contained about 10 % of the 1,3-isomer as determined by NMR according to Serdarevith (44). DACPME, DiC₂OCOC₉, DiC₈ and IpOCOC₉ were synthesized and purified according to previously described principles (21). The 1,2-dioctanoyl-3-o-benzyl-sn-glycerol derivative constituting the ultimate precursor of DiC₈ was > 98 % isomerically pure. However, due to rapid acyl migration during the chromatographic step employed for purification of the deblocked DiC₈, the final preparation predominantly employed in the experiments was found to consist of approximately 30 % of sn-1,2-dioctanoyl-glycerol and 70 % of sn-1,3-dioctanoyl-glycerol. The chemical purity of the compounds was at least 99 % as determined by GLC and HPLC (21).

The procedure followed for examination of human leukocyte histamine release is described elsewhere (6, 7, 9). In brief, venous blood was

harvested from healthy volunteers into EDTA-containing B-D vacutainer tubes. After allowing the red cells to sediment at gravity for 45-60 min at room temperature in a mixture of glucose and dextran-EDTA, the leukocyte-rich plasma layer was recovered and the cells were washed in HEPES-buffer containing 0.3 g human serum albumin (Sigma) per liter. The leukocytes were resuspended in HEPES-ACM buffer (also containing 0.6 mM Ca^{2+} and 1.0 mM MgCl_2). 120 μl of the cell suspension was portioned into tubes and 1 ml of a sonicated sample of the appropriate glycerol derivative or phorbol ester, or the corresponding vehicle prepared in the appropriate medium, was added. After incubation for approximately 5 min at 37°C, 120 μl of the appropriate vehicle or stimulus at the indicated final concentration was added. The number of leukocytes per tube varied with the experiment; normally each tube contained between 10^4 and 10^5 cells (based on measurements of total cellular histamine content and on the figure 1-2 μg histamine/ 10^6 basophils (for ref. see 9)).

The release reaction was allowed to proceed for 40 min and was terminated by immersing the tubes in ice. Each experimental sample was run in duplicate. Histamine secreted into the medium and that recovered from the cell pellet after addition of perchloric acid was extracted and assayed spectrophotofluorimetrically after condensation with o-phthalaldehyde either manually as described previously (6, 7, 20) or by an autonomic procedure (45). In the last-mentioned case the release experiments were performed in the presence of 0.03 % human albumin instead of the 0.3 % used normally.

Means were calculated from duplicates, and the amount of histamine released into the supernatant was expressed as per cent of the total cellular histamine content after correction for spontaneous release. The expression *expected histamine release* (in Figs. denoted E) is the sum of the net histamine release induced by two agents examined separately; the release actually determined in the simultaneous presence of the two agents is denoted the *recorded release* (in Figs. denoted by R).

RESULTS

Modulatory effects of IpOCOC₉ on histamine release induced by anti-IgE, A23187, FMLP or PMA

If leukocytes were exposed to 21 μM , 69 μM or 206 μM IpOCOC₉ 5 min before challenge with the putative calcium ionophore (A23187) the histamine release recorded was either higher or lower than the release which was expected on additive grounds (Fig. 1A). Similarly, pretreatment of cells with IpOCOC₉ apparently could either augment or reduce the release induced by anti-IgE (Fig. 1B). However, in the majority of experiments, pretreatment with IpOCOC₉ led to reduced release at triggering with FMLP (Fig. 1C), but more consistently enhanced release when PMA was employed as a secretagogue (Fig. 1D). Note that these modulatory effects of IpOCOC₉ in many cases could be observed at 69 μM (and sometimes even at 21 μM), i.e., concentrations at which the compound by itself did not normally trigger histamine release (9).

We examined the linear correlation between the degree of enhancement/inhibition recorded in each experiment (i.e. the ratio R/E of recorded release (R) to expected release (E)) and the release induced by either the compound A23187 or the agent IpOCOC₉ separately. This examination showed that a significant negative correlation exists between R/E and A23187-induced release (Fig. 2A). However, no correlation was seen between R/E and IpOCOC₉-induced release (Fig. 2B). Thus, for a cell population responding with a high degree of release to A23187, the pretreatment with IpOCOC₉ leads to a lower than expected degree of release (i.e. to an inhibition), whereas for a cell population responding only weakly to A23187, the presence of IpOCOC₉ markedly augments secretion. If one accepts the concept that A23187 triggers histamine release mainly by acting as a calcium ionophore (27), then IpOCOC₉ in these experiments acts as one would expect from a compound stimulating protein kinase C.

When PMA was employed as a secretagogue, the degree of enhancement/inhibition

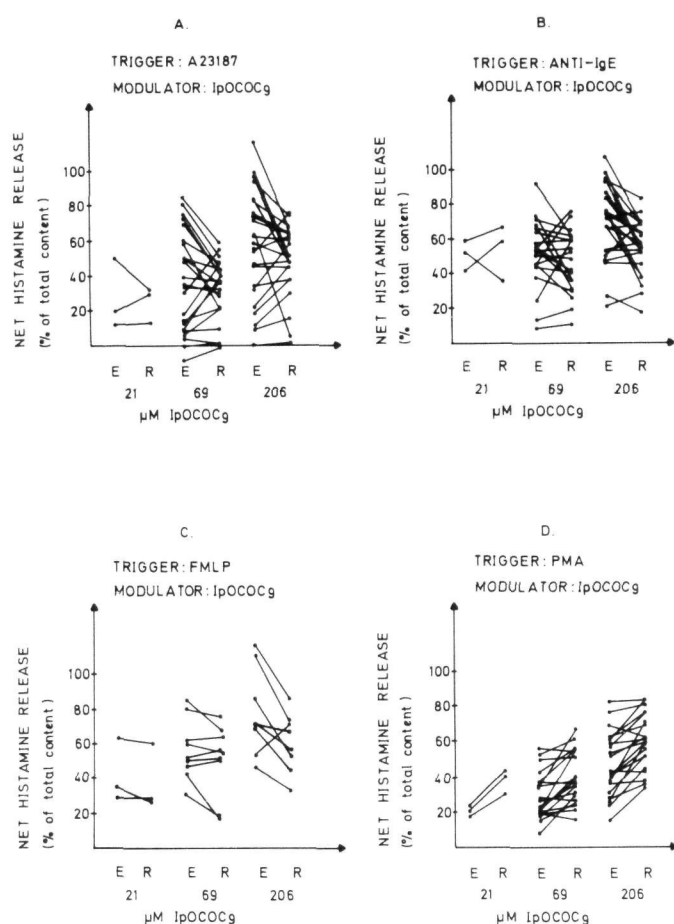


Fig. 1. Effects of IpOCOC₉ (21, 69 and 206 μ M) on leukocyte histamine release induced by A23187 (0.06 μ M or 0.2 μ M; Frame A), anti-IgE (dilution 1:10,000; Frame B), FMLP (1 μ M; Frame C) or PMA (160 nM; Frame D). The figure shows for each examined concentration of IpOCOC₉ the expected release (E) and the actually recorded release (R) from cells exposed to IpOCOC₉ approximately 5 min before the indicated challenge.

of release recorded in the presence of IpOCOC₉ (i.e. the ratio R/E) was now negatively correlated to the release induced by IpOCOC₉ (linear correlation coefficient $r = -0.62$, $n = 16$ at IpOCOC₉ 206 μ M), but not to the release induced by PMA ($r = -0.24$ at IpOCOC₉ 69 μ M, $n = 16$, and $r = -0.45$ at IpOCOC₉ 206 μ M, $n = 16$). Thus, IpOCOC₉ showed the highest degree of augmentation of PMA-induced release in cell populations responding only weakly to IpOCOC₉ itself. In this respect IpOCOC₉ acts as one would expect from a compound increasing the intracellular calcium concentration.

Modulation of anti-IgE-induced release by

IpOCOC₉ showed a negative correlation of borderline significance to the release induced by anti-IgE when examined at either 69 μ M IpOCOC₉ ($r = -0.50$, $n = 22$) or when examined at 206 μ M IpOCOC₉ ($r = -0.48$, $n = 22$) and similarly to the release induced by IpOCOC₉ ($r = -0.46$ at 69 μ M and $r = -0.39$ at 206 μ M).

Modulatory effects on histamine release induced by anti-IgE, A23187, FMLP and PMA of the IpOCOC₉ analogue, DACPME

The compound DACPME is structurally related to IpOCOC₉ but fails to induce basophil histamine release at a concentration where IpOCOC₉ is an effective secretagogue (9). However, as shown in Fig. 3, pretreatment of leukocytes with DACPME leads to consistent enhancement of anti-IgE-induced (Frame B) and PMA-induced (Frame D) histamine release, whereas FMLP-triggered release is not markedly influenced by the compound (Frame C). DACPME enhances the release induced by a low concentration of A23187 but reduces that triggered by a somewhat higher concentration of the drug (Frame A).

Modulatory effects on histamine release induced by anti-IgE, A23187, FMLP and PMA of the glycerol derivative, DiC₂OCOC₉

The compound DiC₂OCOC₉ is structurally related to IpOCOC₉. Therefore, we examined the secretagogue as well as the modulatory actions of this compound in comparison with those of IpOCOC₉. DiC₂OCOC₉ induced only marginal degrees of histamine release at 206 μ M ($6.9 \pm 4.5\%$, mean $\pm$ SEM, $n = 14$) and at 300 μ M ($6.6 \pm 1.3\%$, $n = 18$), whereas at 690 μ M $47.9 \pm 24.6\%$ ($n = 9$) of the total histamine content was released. Modulatory effects of DiC₂OCOC₉ were therefore examined at 30, 100 and 300 μ M. The results given in Fig. 4 show that DiC₂OCOC₉ at 30 μ M and at 100 μ M consistently enhanced A23187-induced release, whereas at 300 μ M both enhancement and inhibition were recorded (Frame A), that 300 μ M DiC₂OCOC₉ consistently reduced (but did not enhance) anti-IgE-induced release

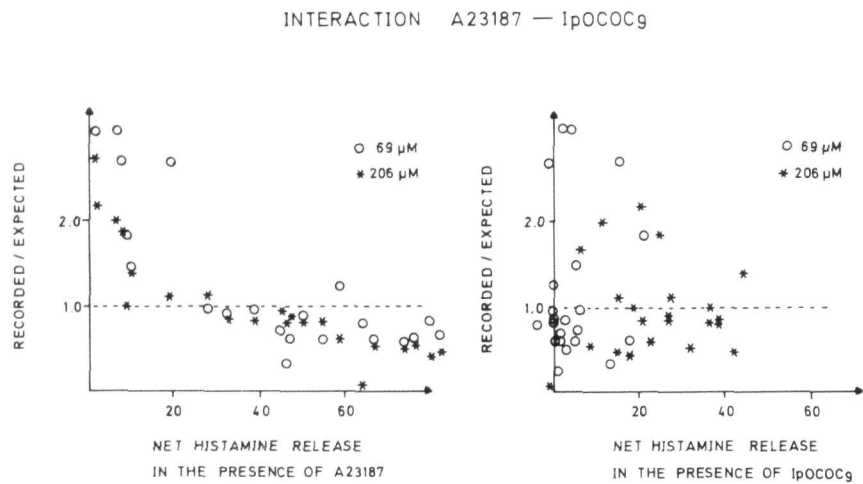


Fig. 2. Linear correlation between degree of enhancement/inhibition of histamine release recorded in the simultaneous presence of both compound A23187 and IpOCOC₉ (i.e. the ratio R/E of recorded (R) release to expected (E)) and the release induced by A23187 separately (Frame A) and that induced by IpOCOC₉ separately (Frame B). Concentrations of IpOCOC₉ employed were either 69 μM or 206 μM as indicated. The coefficients of correlation determined were $r = -0.63$, $n = 21$ at 69 μM IpOCOC₉ and $r = -0.86$, $n = 22$ at 206 μM IpOCOC₉ (Frame A) and $r = 0.07$, $n = 22$ at 69 μM IpOCOC₉ and $r = -0.15$, $n = 23$ at IpOCOC₉ at 206 μM IpOCOC₉ (Frame B).

(Frame B) and FMLP-induced release (Frame C), and that PMA-induced release was either enhanced or reduced in the presence of DiC₂OCOC₉ (Frame D). Thus, DACPME, IpOCOC₉ and DiC₂OCOC₉ seem to differ qualitatively in their modulatory actions on anti-IgE- and on PMA-induced release.

When the correlation between the degree of enhancement/inhibition by DiC₂OCOC₉ of A23187-induced release (i.e. ratio R/E) and the degree of release induced by A23187 (or by DiC₂OCOC₉) was examined separately, a negative correlation was found between R/E and degree of release induced by A23187 when examined at 100 μM DiC₂OCOC ($r = -0.86$, $n = 6$); i.e., under these circumstances DiC₂OCOC₉ acted as one would expect from an

agent with protein kinase C activating properties. On the other hand, when the ratio R/E for the combination DiC₂OCOC₉ and PMA was correlated to the release induced by either of these compounds separately, there was a significant negative correlation between R/E and PMA-induced release ($r = -0.58$, $n = 11$) but not between R/E and DiC₂OCOC₉-induced release ($r = -0.37$, $n = 11$). Admittedly, the release induced by DiC₂OCOC₉ is low in these experiments ($8.8\% \pm 1.4\%$) making correlations of R/E to the release induced by the compound equivocal. However, the point of concern in this case is that a low degree of PMA-induced release could be synergistically enhanced by DiC₂OCOC₉ suggesting that the latter compound now acted by in some way increasing the intracellular concentration of free Ca²⁺.

Table 1

Net histamine release (% of total content) induced by the indicated concentration (in μM for the diacylglycerols and in nM for PMA) of OAG, DiC₈ and PMA. The figures given are means ± SEM from indicated number of experiments

	OAG			DiC ₈		PMA	
μM	69	206	21	69	206	1.6	16
Mean	3.7	24.1	0.0	16.5	40.8	2.6	23.8
SE	2.6	4.6	0.8	4.2	7.1	2.1	7.6
(n)	(6)	(16)	(6)	(9)	(11)	(11)	(8)

Modulatory effects on histamine release triggered by anti-IgE, A23187, FMLP and PMA by the synthetic protein kinase C activators OAG and DiC₈ and by PMA

The protein kinase C activators OAG, DiC₈ and PMA were also examined for modulatory effects on histamine release induced by the above-mentioned secretagogues (i.e. anti-IgE,

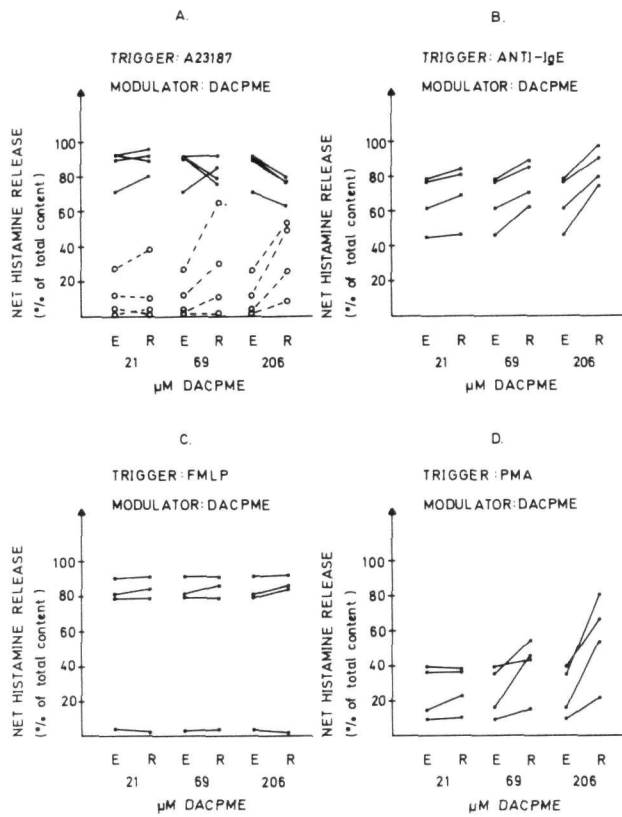


Fig. 3. Effects of DACPME (21, 69 and 206 μ M) on leukocyte histamine release induced by A23187 (0.06 μ M open symbols or 0.2 μ M closed symbols; Frame A), anti-IgE (dilution 1:10,000; Frame B), FMLP (1 μ M; Frame C) or PMA (160 nM; Frame D). The figure shows for each examined concentration of DACPME the E and the R for cells exposed to DACPME approximately 5 min before challenge.

A23187, FMLP and PMA). The secretagogue efficacies of these putative protein kinase C activators are given in Table 1. The biologically inactive isomer of PMA, 4 α -PMA did not induce detectable histamine release even at 100 nM (net release $1.3\% \pm 0.9\%$ mean $\pm$ SEM; $n = 3$).

The results given in Fig. 5 show that OAG, in a substantial part of the experiments, markedly enhanced A23187-induced release; however, occasionally inhibition of release could be observed (Frame A), that OAG marginally reduced anti-IgE-induced release (Frame B) and FMLP-induced release (Frame C), and that OAG, if anything, slightly enhanced PMA-induced histamine release (Frame D). DiC₈ affected secretagogue-induced release, both qualitatively and quantitatively, in the same way as OAG (results not shown). The prepara-

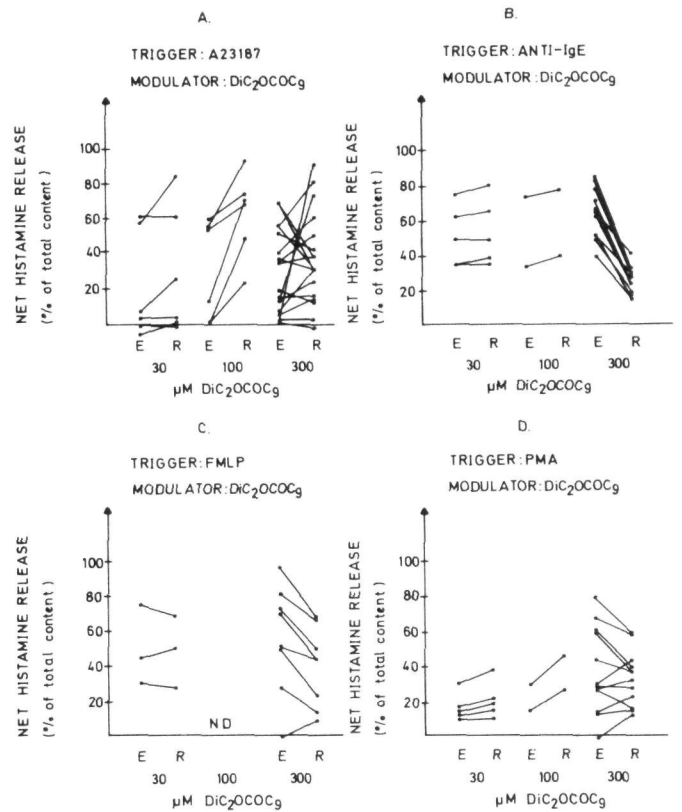


Fig. 4. Effect of DiC₂OCOC₉ (30, 100 and 300 μ M) on leukocyte histamine release induced by A23187 (0.2 μ M; Frame A), anti-IgE (dilution 1:10,000; Frame B), FMLP (1 μ M; Frame C) or PMA (160 nM; Frame D). The figure shows for each examined concentration of DiC₂OCOC₉ the E and the R from cells exposed to DiC₂OCOC₉ approximately 5 min before the indicated secretagogue.

tion of DiC₈ employed in these experiments was a racemic mixture of sn-1,2-dioctanoyl-glycerol and sn-1,3-dioctanoyl-glycerol. However, a few experiments were performed with highly purified fresh preparations of the pure enantiomeric forms sn-1,2-dioctanoyl-glycerol and sn-1,3-dioctanoyl-glycerol. Unexpectedly, both enantiomers were equally effective in inducing basophil histamine release and in modulating the release induced by the secretagogues employed (results not shown). Thus, in these experiments the sn-1,3-dioctanoylglycerol showed biological activity similar to that of sn-1,2-dioctanoyl-glycerol.

Finally, the modulatory effects on secretagogue-induced release of pretreatment of the cells with a low concentration of PMA were examined. Fig. 6 shows the results. As expected, a short pretreatment of the cells with a low

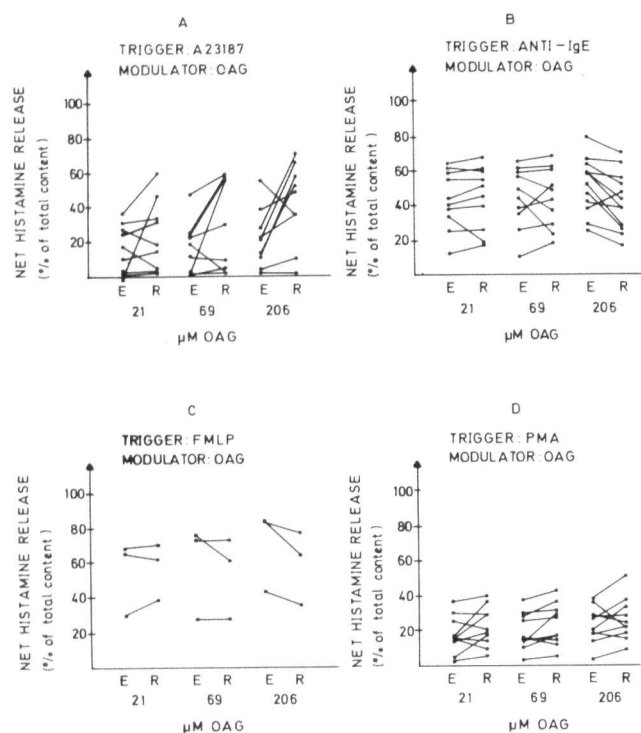


Fig. 5. Effects of OAG (21, 69 and 206 μ M) on leukocyte histamine release induced by A23187 (0.2 μ M; Frame A), anti-IgE (dilution 1:10,000; Frame B), FMLP (1 μ M; Frame C) or PMA (160 nM; Frame D). The figure shows for each examined concentration of OAG the E and the R from cells exposed to OAG approximately 5 min before the indicated secretagogue.

concentration of PMA markedly augmented the release triggered by A23187 (Frame A). The phorbol ester sometimes (at 1.6 nM PMA) enhanced but more regularly (at 16 nM PMA) reduced anti-IgE-induced release. Regularly it did not markedly affect FMLP-induced release. Pretreatment of the leukocytes with a low concentration of PMA (1.6 nM), which in itself did not trigger histamine release, did not affect histamine secretion induced by the ensuing challenge with the 160 nM concentration of PMA (Frame D). However, pretreatment of cells for 5 min with 16 nM PMA (a treatment which, as such, induced release of 22.9% of the total histamine content) led to a lower degree of release than expected at triggering with the 160 nM concentration of PMA. Whether this is due to a real inhibition of release or to a technical artefact due to the design of the experiment is not clear. Anyhow, it should be noted, as described above, that when a similar pretreatment of cells was performed with a diacyl-

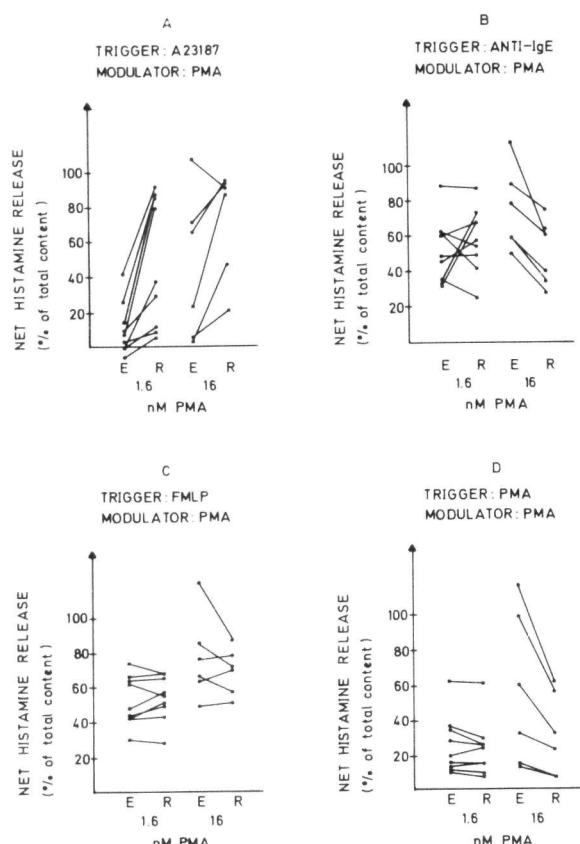


Fig. 6. Effects of PMA (1.6 or 16 nM) on leukocyte histamine release induced by A23187 (0.2 μ M; Frame A), anti-IgE (dilution 1:10,000; Frame B) FMLP (1 μ M; Frame C) or PMA (160 nM; Frame D). The figure shows for each examined concentration of PMA the expected release (E) (i.e., the sum of the release induced by the low concentration of PMA used for pretreatment of the cells and that triggered by the secretagogue separately) and the actually recorded release (R) from cells exposed to PMA approximately 5 min before the indicated secretagogue.

glycerol, enhancement and not inhibition of PMA-triggered release was recorded.

DISCUSSION

Human basophil degranulation (with a concomitant release/generation of various allergic mediators) can be triggered by an array of agents. It is clear that these secretagogues, according to their functional characteristics, can be grouped into different categories including (i) $Fc\epsilon$ -receptor dependent secretagogues (23, 30), (ii) agents which like FMLP, C5a, and pepstatin act on specific univalent membrane receptors (31, 46), (iii) putative calcium ionophores like A23187 (27) and ionomycin, (iv) the

phorbol esters (41, 42), (v) some polycations like polylysine (19) and the major basic protein of eosinophils (35), and (vi) agents which like mannitol (17, 18) trigger release by increasing osmolarity.

The varying characteristics of action of these secretagogues, as well as reports on differential effects of various modulators (12, 30), support the notion that the stimulus-response pathway may vary for each group of secretagogues. Thus, a membrane-bound serine protease activity has been implicated in Fc ϵ -receptor-dependent histamine release (46). Moreover, the phorbol ester-induced triggering would be expected to be due to a direct activation of a protein kinase C, and the action of mannitol is suggested to involve changes in phospholipid metabolism (18). In analogy with the action of FMLP with respect to neutrophil activation (e.g. 10, 15, 24), triggering of the basophil with this agent would be expected to include as an early step a G-protein dependent phospholipase C activation and a concomitant generation from phosphatidylinositol-4,5-bisphosphate of the second messengers inositol trisphosphate (IP₃) and sn-1,2-diacylglycerol (DAG). Conforming to such a view, FMLP-, but not anti-IgE-induced basophil histamine release was recently reported to be sensitive to pertussis toxin treatment of the cells (40, 47, and Bergstrand et al., unpublished). However, purified human basophils are not easy to obtain in reasonable quantities; therefore there are few direct biochemical studies on the pathways for signal transduction initiated by the various secretagogues (cf 32).

The stimulus-secretion relationships have been more closely examined in cultured rat basophilic leukemia (2H3) cells. These studies indicate that the Fc ϵ -receptor-mediated triggering also involves substantial hydrolysis of membrane inositol phospholipids (4, 29), a process which could constitute part of the calcium signal (3, 4). However, the calcium signal in these cells is mainly formed by influx of extracellular Ca²⁺ (3); the basis for this influx is not yet defined. On the other hand, triggering of the cells by A23187 or ionomycin, agents generally thought to act as calcium ionophores (27, 32), results in a low degree of hydrolysis of inositol phospholipids

(40), but recent data indicate that this is mandatory for their actions as secretagogues (28).

In a previous report we have shown that the glyceride derivative IpOCOC₉ is a secretagogue for human basophils with a mode of action which closely resembles that of hyperosmolar mannitol and also in many respects show similarities to that of PMA (9). Moreover, IpOCOC₉ (but not its structural analogue DACPME, which does not trigger basophil histamine release) at 23–206 μ M effectively augments the Ca²⁺- and phosphatidylserine-dependent cytosolic histone III-S kinase activity of human polymorphonuclear leukocytes (H. Olsson & H. Bergstrand, manuscript in preparation). Therefore, we suggested that IpOCOC₉ acts as a basophil secretagogue by stimulating a protein kinase C activity. However, inhibitor studies suggest that this putative protein kinase C activity is not identical to that presumably activated by PMA (8). Since PMA has been reported to interfere with basophil histamine release triggered by other secretagogues in a characteristic way (42), we examined the modulatory effects on secretagogue-induced release of IpOCOC₉ and of DACPME in comparison with those of PMA. In these experiments we also employed the synthetic diacylglycerol derivatives OAG and DiC₈ and a structural analogue of IpOCOC₉,

Table 2

Schematic representation of the modulatory effects exerted by DACPME, IpOCOC₉, DiC₂OCOC₉, OAG, DiC₈ and PMA on basophil histamine release induced by anti-IgE, A23187, FMPL and PMA.

Modulator	Secretagogue			
	anti-IgE	A23187	FMLP	PMA
IpOCOC ₉	↑ ↓	↑ ↓	↓	↑
DACPME	↑	↑ ↓	O	↑
DiC ₂ OCOC ₉	↓	↑ ↓	↓	↓
OAG	(↑) ↓	↑ (↓)	(↓)	↑ (↓)
DiC ₈	(↑) ↓	↑	O	↑
PMA	↑ ↓	↑	(↓)	

↑ = enhanced release in the presence of the modulator; ↓ = reduced release; O = no influence on release. Symbols in parenthesis denote marginal effects.

i.e. $\text{DiC}_2\text{OCOC}_9$. Table 2 summarizes in schematic form our findings depicted in detail in Figs. 1–6.

The following are salient recordings: (i) The modulatory actions of IpOCOC_9 are qualitatively and quantitatively almost identical whether examined at a non-secretagogue concentration (69 μM) or at a secretagogue concentration (206 μM). Furthermore, the modulatory actions of IpOCOC_9 in many respects mimic those of the non-secretagogue DACPME. Thus, the modulatory actions of IpOCOC_9 seem to be independent of its secretagogue activity. (ii) The modulatory effects of IpOCOC_9 on A23187- and anti-IgE-induced release (but apparently not those on FMLP- or PMA-induced release) include both enhancing and inhibiting actions. Of these, the enhancing activity on A23187-induced release from correlation analysis can be characterized as being functionally similar to what one would expect from a protein kinase C activator, whereas the enhancing activity of the agent on PMA-induced release according to a similar analysis would be expected to be due to increased cytosolic Ca^{2+} . That this pattern of modulatory action recorded for IpOCOC_9 is not a trivial one follows from the fact that the patterns of action of the closely related compounds DACPME and $\text{DiC}_2\text{OCOC}_9$ are qualitatively different. Thus, contrary to IpOCOC_9 , the former compound does not reduce anti-IgE-triggered release, whereas the latter compound does not enhance anti-IgE-induced release. Moreover, again contrary to IpOCOC_9 , $\text{DiC}_2\text{OCOC}_9$ is clearly capable of reducing PMA-triggered histamine release. The reasons for these interesting phenomenological observations are presently completely unknown but certainly warrant further studies. (iii) The modulatory actions of IpOCOC_9 are in many respects mimicked by the two examined synthetic diacylglycerols OAG and DiC_8 and by low concentrations of PMA. This would suggest that IpOCOC_9 could act not only as a secretagogue but also as a modulator by direct or indirect interference with a protein kinase C activity.

However, each of the examined glyceride derivatives displays certain distinct characteris-

tics of action not shared by the others. For example, the stimulatory influence of IpOCOC_9 on PMA-induced release tends to be more expressed than those of OAG and DiC_8 ; conversely the stimulation of A23187-induced release exerted by OAG and DiC_8 seems more expressed than that of IpOCOC_9 . Moreover, there is no indication that pretreatment of cells with low concentrations of PMA could lead to enhanced release at an ensuing challenge with a higher concentration of PMA; such a situation is often observed for OAG and DiC_8 as well as for IpOCOC_9 . These data conform to and extend a number of previous observations in other systems pointing to qualitative differences in actions between synthetic diacylglycerols and PMA. Thus, OAG was found to induce phosphorylation of only 9 of 14 proteins phosphorylated at exposure of HL-60 promyelocytic cells to PMA (25). OAG and PMA were found to differ with respect to induction of platelet aggregation and phosphorylation of a 40 kDa protein (1). Some effects of DiC_8 on vasopressin-induced water flow in the bladder could not be mimicked by PMA or vice versa (43). Moreover, DiC_8 completely inhibited binding of labelled phorbol dibutyrate to intact HL-60 cells, whereas OAG was only partially active in this respect (16). Likewise, OAG was only a partial agonist compared with DiC_8 with respect to release of luteinizing hormone from pituitary cell cultures (14). The activities of DiC_8 and OAG differ from each other and from PMA with respect to phosphatidylcholine biosynthesis and epidermal growth factor binding to HEL-37 cells (33). In the present experiments we found no clearcut qualitative difference in modulatory action between OAG and DiC_8 .

With respect to secretagogue activity, the characteristics of IpOCOC_9 were found to mimic those of hyperosmolar mannitol even more than those of PMA (9). The mechanisms of hyperosmolar mannitol-triggered release are not clarified, but membrane perturbation possibly leading to protein kinase C activation has been discussed (18). Moreover, hyperosmolarity inhibits FMLP- and leukotriene B_4 -triggered neutrophil degranulation and the concomitant actin polymerization (cf 48). The latter process

is associated with a decrease in cell volume; it may depend on protein kinase C activation. PMA-induced increase in cytoskeletal actin is not reduced by hyperosmolarity (48).

In conclusion, the present report defines modulatory characteristics on human leukocyte histamine release of the glycerol derivative IpOCOC₉ which seem to be independent of its secretagogue action(s). In many respects, the characteristics of these modulatory actions mimic those of synthetic diacylglycerols and those of the phorbol ester PMA, tentatively suggesting that they are due to protein kinase C interference. However, the action of IpOCOC₉, as well as that of its structural relatives, DACPME and DiC₂OCOC₉, shows some distinct individual characteristics not shared by the other examined compounds. Whether this could reflect differential interaction with different entities of the protein kinase C family, which from cDNA structures have been identified in various human cells (13, 22, 36–39), is a possibility that would warrant further studies, especially since various forms of protein kinase C apparently may show differences in their degree of activation by diacylglycerols and by phorbol esters (39).

ACKNOWLEDGEMENTS

We are grateful to B. G. Josefsson and G. Kjellin, The Organic Chemistry Unit, AB Draco, for the synthesis of OAG and to Mrs. M. Broman and I. Källén for skilful secretarial assistance.

REFERENCES

1. Ashby, B., Kowalska, M. A., Wernick, E., Rigmaiden, M., Daniel, J. L. & Smith, J. B.: Differences in the mode of action of 1-oleoyl-2-acetyl-glycerol and phorbol ester in platelet activation. *J. Cyclic Nucleotide Protein Phosphor Res.* 10, 473–483, 1985.
2. Ashendel, C. L.: The phorbol ester receptor: A phospholipid-regulated protein kinase. *Biochim. Biophys. Acta.* 822, 219–242, 1985.
3. Beaven, M. A., Rogers, J., Moore, J. P., Hesketh, T. R., Smith, G. A. & Metcalfe, J. C.: The mechanism of the calcium signal and correlation with histamine release in 2H3 cells. *J. Biol. Chem.* 259, 7129–7136, 1984.
4. Beaven, M. A., Moore, J. P., Smith, G. A., Hesketh, T. R. & Metcalfe, J. C.: The calcium signal and phosphatidylinositol breakdown in 2H3 cells. *J. Biol. Chem.* 259, 7137–7142, 1984.
5. Bell, R. M.: Protein kinase C activation by diacylglycerol second messengers. *Cell* 45, 631–632, 1986.
6. Bergstrand, H., Björnson, A., Frick, I.-M., Lundquist, B. & Petersson, B. Å: Anti-IgE-induced histamine release from human basophilic leukocytes: Inhibition depends on nature and concentration of inhibitor and secretagogue. *Allergy* 37, 503–512, 1982.
7. Bergstrand, H., Björnson, A., Lundquist, B., Nilsson, A. & Brattsand, R.: Inhibitory effect of glucocorticosteroids on anti-IgE-induced histamine release from human basophilic leukocytes: Evidence for a dual mechanism of action. *Allergy* 39, 217–230, 1984.
8. Bergstrand, H., Lundquist, B. & Michelsen, P.: Human leukocyte histamine release induced by sn-1,2-isopropylidene-3-decanoyl-glycerol (IpOCOC₉). Effects of putative protein kinase C inhibitors. (Manuscript submitted).
9. Bergstrand, H., Lundquist, B. & Michelsen, P.: Induction of human basophil histamine release by a novel protein kinase C activator, sn-1,2-isopropylidene-3-decanoyl-glycerol (IpOCOC₉). Partial characterization of secretagogue characteristics. *Eur. J. Haematol.* (in press).
10. Bradford, P. G. & Rubin, R. P.: Characterization of formyl-methionyl-leucyl-phenylalanine stimulation of inositol trisphosphate accumulation in rabbit neutrophils. *Mol. Pharmacol.* 27, 74–78, 1985.
11. Buchnea, D.: Synthesis of C-18 mixed diacyl-sn-glycerol enantiomers. *Lipids* 6, 734–739, 1971.
12. Conroy, M. C., Kennedy, J. A. & Thuesen, D. O.: Differential inhibition of histamine release from human basophils induced by antigen, anti-IgE or N-formyl-l-methionyl-l-leucyl-l-phenylalanine. *Int. Arch. Allergy Appl. Immunol.* 77, 222–224, 1985.
13. Coussens, L., Parker, P. J., Rhee, L., Yang-Feng, T. L. & Ullrich, A.: Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. *Science* 233, 859–866, 1986.
14. Davis, R. J., Ganong, B. R., Bell, R. M. & Czech, M. P.: Structural requirements for diacylglycerols to mimic tumor-promoting phorbol diester action on the epidermal growth factor receptor. *J. Biol. Chem.* 260, 5315–5322, 1985.
15. Di Virgilio, F., Vicentini, L. M., Treves, S., Riz, G. & Pozzan, T.: Inositol phosphate formation in fet-Leu-Phe-stimulated human neutrophils does not require an increase in the cytosolic free Ca²⁺ concentration. *Biochem. J.* 229, 361–367, 1985.
16. Eberling, J. G., Vandenbark, G. R., Kuhn, L. J., Ganong, B. R., Bell, R. M. & Nidel, J. E.: Diacylglycerols mimic phorbol diester induction of leukemic cell differentiation. *Proc. Natl. Acad. Sci. USA* 82, 815–819, 1985.
17. Eggleston, P. A., Kagey-Sobotka, A. & Lichtenstein, L. M.: A comparison of the osmotic activation of basophils and human lung mast cells. *Ann. Rev. Respir. Dis.* 135: 1043–1048, 1987.
18. Findlay, S. R., Dvorak, A. M., Kagey-Sobotka, A. &

- Lichtenstein, L. M.: Hyperosmolar triggering of histamine release from human basophils. *J. Clin. Invest.* 67, 1604-1613, 1981.
19. Foreman, J. C. & Lichtenstein, L. M.: Induction of histamine secretion by polycations. *Biochim. Biophys. Acta.* 629, 587-603, 1980.
20. Grant, J. A., Settle, L., Whorton, E. B. & Dupree, R.: Complement-mediated release of histamine from human basophils. II. Biochemical characterization of the reaction. *J. Immunol* 117: 450-456, 1976.
21. Gronowitz, S., Herslöf, B. Ohlson, R. & Töregård, B.: ORD and CD studies of saturated glycerids. *Chem. Phys. Lipids* 14, 174-188, 1975.
22. Housey, G. M., O'Brian, A., Johnson, M. D., Kirschmeier, P. & Weinstein, I. B.: Isolation of cDNA clones encoding protein kinase C: Evidence for a protein kinase C-related gene family. *Proc. Natl. Acad. Sci. USA* 84, 1065-1069, 1987.
23. Ishizaka, T., Conrad, D. H., Schulman, E. S., Sterk, A. R., Ko, C. G. L. & Ishizaka, K.: IgE-mediated triggering signals for mediator release from human mast cells and basophils. *Fed. Proc.* 43, 2840-2845, 1984.
24. Krause, K. H., Schlegel, W., Wollheim, C. B., Andersson, T., Waldvogel, F. A. & Lew, P. D.: Chemotactic peptide activation of human neutrophils and HL-60 cells. Pertussis toxin reveals correlation between inositol trisphosphate generation, calcium ion transients, and cellular activation. *J. Clin. Invest.* 76, 1348-1354, 1985.
25. Kreutter, D., Caldwell, A. B. & Morin, M. J.: Dissociation of protein kinase C activation from phorbol ester-induced maturation of HL-60 leukemia cells. *J. Biol. Chem.* 260, 5979-5984, 1985.
26. König, B., Di Nitto, P. A. & Blumberg, P. M.: Phospholipid and Ca^{++} dependency of phorbol ester receptors. *J. Cell. Biochem.* 27, 255-265, 1985.
27. Lichtenstein, L. M.: The mechanism of basophil histamine release induced by antigen and the calcium ionophore A23187. *J. Immunol.* 114, 1692-1699, 1975.
28. Lo, T. N., Saul, W. & Beaven, M. A.: The actions of Ca^{2+} ionophores on rat basophilic (2H3) cells are dependent on cellular ATP and hydrolysis of inositol phospholipids. A comparison with antigen stimulation. *J. Biol. Chem.* 262, 4141-4145, 1987.
29. Maeyama, K., Hohman, R. J., Metzger, H. & Beaven, M. A.: Quantitative relationships between aggregation of IgE receptors, generation of intracellular signals, and histamine secretion in rat basophilic leukemia (2H3) cells. *J. Biol. Chem.* 261, 2583-2592, 1986.
30. Marone, G., Kagey-Sobotka, A. & Lichtenstein, L. M.: IgE-mediated histamine release from human basophils: Differences between antigen E- and anti-IgE-induced secretion. *Int. Arch. Allergy Appl. Immunol.* 65, 339-348, 1981.
31. Marone, G., Columbo, M., Soppelsa, L. & Condorelli, M.: The mechanism of basophil histamine release induced by pepstatin A. *J. Immunol.* 133, 1542-1546, 1984.
32. Metzger, H. & Kinet, J.-P.: How antibodies work: focus on Fc receptors. *FASEB J.* 2, 3-11, 1988.
33. Muir, J. G. & Murray, A. W.: Mimicry of phorbol ester responses by diacylglycerols. Differential effects on phosphatidylcholine biosynthesis, cell-cell communication and epidermal growth factor binding. *Biochim. Biophys. Acta.* 885, 176-184, 1986.
34. Nishizuka, Y.: The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature* 308, 693-698, 1984.
35. O'Donnell, M. C., Ackerman, S. J. Gleich, G. J. & Thomas, L. T.: Activation of basophil and mast cell histamine release by eosinophil granule major basic protein. *J. Exp. Med.* 157, 1981-1991, 1983.
36. Ohno, S., Kawasaki, H., Imajoh, S. & Suzuki, K.: Tissue-specific expression of three distinct types of rabbit protein kinase C. *Nature* 325, 161-166, 1987.
37. Ono, Y., Fujii, T., Ogita, K. Kikkawa, U., Igarashi, K. & Nishizuka, Y.: Identification of three additional members of rat protein kinase C family: delta-, epsilon- and zeta-subspecies. *FEBS Lett.* 226, 125-128, 1985.
38. Parker, P. J., Coussens, L., Totty, N., Rhee, L. & Ullrich, A.: The complete primary structure of protein kinase C - the major phorbol ester receptor. *Science* 233, 853-859, 1986.
39. Pelosin, J.-M., Vilgrain, I. & Chambaz, E. M.: A single form of protein kinase C is expressed in bovine adrenocortical tissue, as compared to four chromatographically resolved isoenzymes in rat brain. *Biochem. Biophys. Res. Commun.* 147, 382-391, 1987.
40. Saito, H., Okajima, F., Molski, T. F. P., Sha'afi, R. I., Ui, M. & Ishizaka, T.: Effects of ADP-ribosylation of GTP-binding protein by pertussis toxin on immunoglobulin E-dependent and -independent histamine release from mast cells and basophils. *J. Immunol.* 138, 3927-3934, 1987.
41. Schleimer, R. P., Gillespie, E. & Lichtenstein, L. M.: Release of histamine from human leukocytes stimulated with the tumor-promoting phorbol diesters. *J. Immunol.* 126, 570-574, 1981.
42. Schleimer, R. P., Gillespie, E., Daiuta, R. & Lichtenstein, L. M.: Release of histamine from human leukocytes stimulated with the tumor-promoting phorbol diesters II. Interaction with other stimuli. *J. Immunol.* 128, 136-140, 1982.
43. Schlondorff, D. & Levine, S. D.: Inhibition of vasopressin-stimulated water flow in toad bladder by phorbol myristate acetate, dioctanoylglycerol, and RHC-80267. *J. Clin. Invest.* 76, 1071-1078, 1985.
44. Serdarevich, R.: Glyceride isomerizations in lipid chemistry. *J. Am. Oil. Chem.* 44, 381-393, 1967.
45. Siraganian, P.: An automated continuous flow system for extraction and fluorimetric analysis of histamine. *Anal. Biochem.* 57, 383-394, 1974.
46. Siraganian, R. P. & Hook, W. A.: Mechanism of histamine release by formyl methionine-containing peptides. *J. Immunol.* 119, 2078-2083, 1977.
47. Warner, J. A., Yancey, K. B. & MacGlashan, D. W.: The effect of petussis toxin on mediator release from human basophils. *J. Immunol.* 139, 161-165, 1987.

48. Yassin, R., Shefcyk, J., White, J. R., et al.: Effects of chemotactic factors and other agents on the amounts of action and a 65,000-mol-wt protein associated with the cytoskeleton of rabbit and human neutrophils. *J. Cell. Biol.* *101*, 182-188, 1985.

Address:

Håkan Bergstrand

The Pharmacological Laboratory

Research and Development Department

AB Draco, Box 34,

S-221 00 Lund

Sweden

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.