

Human basophil histamine release is differently affected by inhibitors of calmodulin, diacylglycerol kinase and peptidyl prolyl cis-trans isomerase in a secretagogue specific manner

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To assess the role of calmodulin in human basophil histamine release, we triggered leukocytes with different secretagogues in the presence of putative inhibitors of calmodulin. Calcium ionophore-induced histamine release was reduced or blocked by calmidazolium, CGS 9343B, felodipine, metofenazate, and Ro 22-4839. H 186/86, a felodipine-related dihydropyridine derivative, blocked A23187-but not ionomycin-triggered histamine release, suggesting a difference in the mode of action of these ionophores. In contrast, leukocyte histamine release triggered by the purported protein kinase C (PKC) activator, 1,2-isopropylidene-3-decanoyl-*sn*-glycerol (IpOCOC₉), was enhanced by calmidazolium, CGS 9349B and metofenazate but not affected by felodipine or Ro 22-4839, whereas the response triggered by 4 β -phorbol 12-myristate 13-acetate (PMA) was reduced by metofenazate and Ro 22-4839 but not consistently affected by calmidazolium, CGS 9343B or felodipine. The PMA-induced histamine release was enhanced by H 186/86. Anti-IgE- and FMLP-induced responses were either unaffected or slightly enhanced by the examined calmodulin antagonists. In comparison with the calmodulin antagonists, R 59022, an inhibitor of diacylglycerol kinase, failed to reduce calcium ionophore-triggered histamine release, whereas FK506, an inhibitor of peptidyl prolyl cis-trans isomerase (PPI), reduced *both* anti-IgE- and ionophore-triggered responses. These results indicate that calmodulin constitutes an obligate link in signal transduction pathways leading to human leukocyte histamine release if the trigger is a calcium ionophore but not when responses are induced by anti-IgE, FMLP or PMA; a calmodulin-dependent component may rather balance responses induced by IpOCOC₉. We also conclude that most employed stimuli, including IpOCOC₉, trigger human basophil histamine release through partly distinct pathways.

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Abbreviations: CGS 9343B: 1,3-Dihydro-1-[1-[(4-methyl-4H, 6H-pyrrolo[1,2-a][4,1]-benzoxazepin-4-yl)methyl]-4-piperidinyl]-2H-benzimidazol-2-one (1:1) maleate; DAG: diacylglycerol; FKBP: FK506-binding protein; FMLP: formyl-methionyl-leucyl-phenylalanine; H 186/86: 3,5-pyridinedicarboxylic acid, 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-, ditertbutyl ester; IpOCOC₉: 1,2-isopropylidene-3-decanoyl-*sn*-glycerol; PKC: protein kinase C; PMA: 4 β -phorbol 12-myristate 13-acetate; PPI: peptidyl prolyl cis-trans isomerase; R 59 022: (6-[2-(4[4fluoro-phenyl]-phenylmethylene]-1-piperidinyl)-jethyl]-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one); Ro 22-4839: 1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-4-[[4-(2-methoxyphenyl)-1-piperazinyl]methyl]-isoquinoline maleate (1:1); RBL: rat basophilic leukemia.

Human basophil histamine release can be triggered by various stimuli. Of these, antigen and anti-IgE crosslink high affinity IgE receptors (28, 29) which results in an increase in cytosolic Ca²⁺ levels and membrane-associated protein kinase C (PKC) with no loss of cytosolic PKC activity (47, 48). In rat basophilic leukemia (RBL) cells, IgE receptor-mediated secretion is likewise accompanied by phospholipid breakdown, a rise in cytosolic calcium, and PKC-mediated myosin light and heavy chain phosphorylation (1, 24). Calcium ionophores (22) and

phorbol esters (2, 10, 30, 38, 47) are thought to induce histamine release by directly raising intracellular Ca^{2+} and activating PKC, respectively. Accordingly, PKC inhibitors block phorbol ester-triggered histamine release (21, 31, 44, 48, Bergstrand et al., submitted to *J Pharmacol Exp Ther*). However, in the RBL cell system, the signals triggered by a combination of calcium ionophore and phorbol ester are not the same as those generated by IgE receptor aggregation (9) and IgE-mediated human leukocyte histamine release is not, like PMA-triggered release, blocked by the PKC inhibitor Ro 31-7549 (Bergstrand et al., submitted). Moreover, formyl-methionyl-leucyl-phenylalanine (FMLP) binds to univalent receptors and probably also initiates histamine release through generation of inositol triphosphate and diacylglycerol (DAG) (25, 28, 29, 41, 49). Despite this, responses induced by FMLP are not reduced by PKC inhibitors (21, 48, Bergstrand et al., submitted).

Thus, the biochemical mechanisms involved in basophil degranulation differ with secretagogue (2, 28, 44, 48, 49). Their elucidation will require extensive biochemical studies. Since basophils are difficult to purify in adequate amounts, delineation of signal transduction pathways also has to rely on functional studies of cells in the presence of modulators of specified enzymes (7, 27).

To that end, we reported that 1,2-isopropylidene-3-decanoyl-*sn*-glycerol (IpOCOC₉) triggers human leukocyte histamine release possibly through PKC activation (2, 35). However, the actions of IpOCOC₉ and the prototype PKC agonist, 4 β -phorbol 12-myristate 13-acetate (PMA), differ in some respects and PKC inhibitors affect IpOCOC₉- and PMA-triggered histamine release in different ways (2, Bergstrand et al., submitted). IpOCOC₉ enhances the main PKC activity of PMN leukocytes by replacing either the phorbol ester or the Ca^{2+} and phospholipid requirements (35) and accordingly synergize with A23187 as well as with PMA in triggering histamine release (3). Therefore, IpOCOC₉ perhaps interacts also with proteins other than PKC known to require calcium for activity. Of these, calmodulin has been attributed a main function in human basophil histamine release (27, 44).

With the aim of defining the role of calmodulin in human leukocyte histamine release with special reference to the response triggered by IpOCOC₉, we compared effect of five structurally unrelated, selective inhibitors of calmodulin on leukocyte histamine release triggered by this agent with their effects on responses induced by anti-IgE, calcium ionophores, FMLP, PMA and hyperosmolar mannitol. Moreover, to define the specificity of the effects recorded with these inhibitors, we compared them with effects of inhibitors of DAG kinase (R 59 022 (11)) and

peptidyl prolyl cis-trans isomerase (FK506). DAG kinase, which might influence signal transduction events by phosphorylating DAG to phosphatidic acid, contains E-F hand motifs typical of Ca^{2+} -binding proteins like calmodulin; the activity of DAG kinase is augmented by calcium (36). Although peptidyl prolyl cis-trans isomerases (PPI) are considered the main target of the immunosuppressive agents cyclosporine A and FK506 (5, 14, 19, 39, 43)), these agents also interfere with calmodulin and the drug-immunophilin complexes bind the phosphatase calcineurin (23); both compounds were recently reported to inhibit calcium ionophore- and/or anti-IgE-induced human basophil and RBL cell histamine release (6, 12, 17).

Material and methods

The following compounds were purchased as indicated: A23187, calmidazolium (1-[bis(4-chlorophenyl)methyl]-3-[2,4-dichloro- β -(2,4-dichlorobenzoyloxy)phenethyl]-imidazolium chloride), formyl-methionyl-leucyl-phenylalanine (FMLP), D-mannitol and 4 β -phorbol 12-myristate 13-acetate (PMA) from Sigma; ionomycin from Calbiochem, and R 59 022 (6-[2-(4-[(4-fluoro-phenyl)phenyl-methylene]-1-piperidinyl)ethyl]-7-methyl-5H-thiazolo[3,2-a]-pyrimidin-5-one) from Janssen. Compound CGS 9343B (1,3-Dihydro-1-[1-[(4-methyl-4H,6H-pyrrolo[1,2-a]-[4,1]benzoxazepin-4-yl)methyl]-4-piperidinyl]-2H-benzimidazol-2-one (1:1) maleate) was kindly provided by Dr J. Watthey, Ciba Geigy Corp., Summit NJ, USA; felodipine and H 186/86 (3,5-pyridinedicarboxylic acid, 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-, ditertbutyl ester) by Astra Hässle, Mölndal, Sweden; FK506 by K. Inokuchi, Fujisawa Pharmaceutical, Osaka, Japan; metofenazate difumarate by Egis Pharmaceuticals, Budapest, Hungary; and Ro 22-4839 (1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-4-[[4-(2-methoxy-phenyl)-1-piperazinyl]-methyl]isoquinolone maleate (1:1)) by E. Johansson, Roche, Stockholm, Sweden. The compound 1,2-isopropylidene-3-decanoyl-*sn*-glycerol (IpOCOC₉) was synthesized as described (2). Fig. 1 shows the structures of the examined compounds.

The preparation of the anti-IgE utilized and the sources of other reagents are previously specified (2, 3).

The procedure for examining human leukocyte histamine release is that described elsewhere (2, 3). In short, venous blood from healthy subjects was collected in EDTA-containing vacutainer tubes and mixed with dextrane-glucose solution. The red cells were allowed to sediment at gravity and the leukocytes were harvested and washed in HEPES buffer without added Ca^{2+} and Mg^{2+} . The cells were resuspended in HEPES buffer containing 0.6 mM

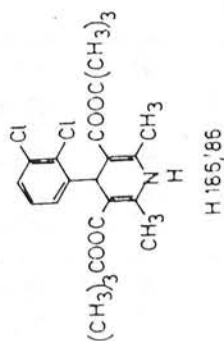
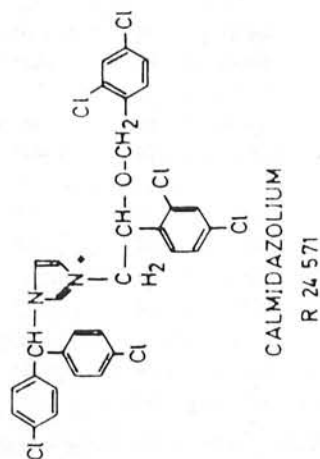
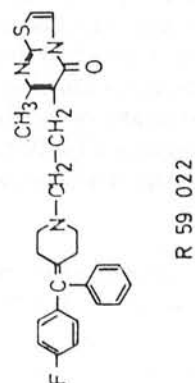
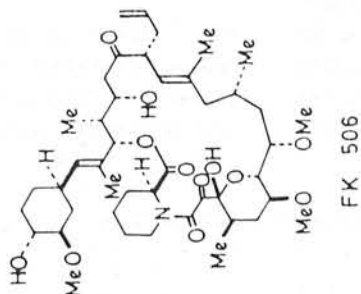
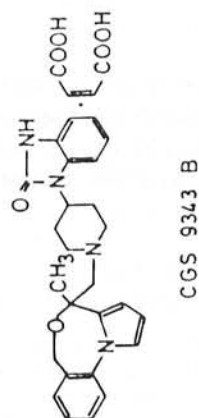
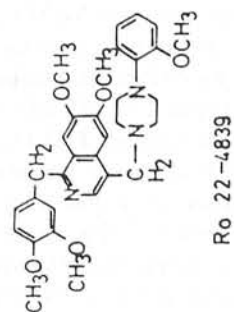
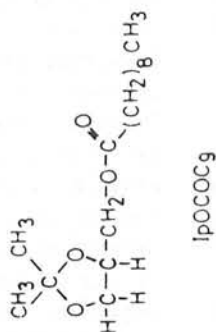
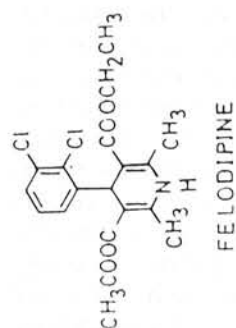


Fig. 1. Structures of IpOCOC₉ and employed modulators.

Ca^{2+} and 1.0 mM Mg^{2+} and 0.03% human serum albumin (denoted buffer HACM). 120 μl of the cell suspension was portioned into each reaction tube. One ml of the examined modulator (dissolved or uniformly suspended in HACM) was added. Control tubes received vehicle. Following 5 min incubation at 37°C, 120 μl of the pertinent stimulus was added. The stimulus concentrations are given in the legend to Fig. 2 and in Table 1. Two concentrations of A23187 were employed: 0.06 μM and 0.2 μM . The former induces *per se* a low degree of release; it is therefore suitable for assessment of any synergism in action with that of the employed compounds. At challenge with hyperosmolar mannitol, the cells were incubated with 500 μl inhibitor and challenge was performed with 500 μl 1.67 M mannitol in HACM also containing the pertinent inhibitor. The concentrations given of inhibitors and secretagogues are those prevailing in the reaction mixture.

The release process was allowed to proceed for 40 min. Each assay was run in duplicate. Histamine secreted into the medium and that obtained from the cell pellet by extraction with PCA were assayed spectrophotofluorimetrically after condensation with o-phthalaldehyde by an automated procedure essen-

tially as described elsewhere (3, 40). Means of the duplicates of each assay were calculated and the histamine released into the supernatant was expressed as per cent of total cellular histamine content after correction for spontaneous release (rarely exceeding 5%). The expected histamine release (denoted **E**) is the sum of the net release induced by the secretagogue employed and that induced by the examined modulator *per se* as determined separately. As can be seen from Table 1, the latter is generally negligible. The net release actually recorded in the presence of both the secretagogue and the inhibitor is denoted the recorded release (**R**). The effect of each compound was examined in at least six different experiments: statistical significance of any effect was assessed with the aid of Wilcoxon's signed rank test on paired observations.

Results

Effects of CGS 9343B

Fig. 2 illustrates the results obtained at experiments with CGS 9343B. From these results the mean degree of recorded histamine release can be calculated and expressed as percentage of the mean degree of

Table 1. Effects of indicated modulators on leukocyte histamine release triggered by the indicated secretagogues

	Conc (μM)	Net release $\pm$ SEM (n)	Histamine release (% of expected)							
			A23187		Anti-IgE	FMLP	Ionomycin	IpOCOC ₉	Mannitol	PMA
			0.06 μM	0.2 μM						
Calmidazolium	2.1		62	73*	104 (2)	101 (2)	79*	98		
	6.9	12.6 $\pm$ 9.3 (8)	64*	56*	92	106	72*	138*	108	100
CGS 9343B	6.9	0.7 $\pm$ 0.9 (7)	96	80*	122*	110	104	129*	92	96
	69	3.3 $\pm$ 1.4 (8)	28*	17*	119	122*	41*	205*	88	100
Felodipine	0.1		65 (2)	78 (2)	100 (2)	107 (2)	127 (2)	126 (2)		100 (2)
	1		59 (3)	63 (3)	77 (3)	75 (3)	71 (3)	89 (3)	107	88 (3)
	10	3.3 $\pm$ 1.1 (4)	36*	13*	93	55 (4)	18*	123 (4)	115	86 (4)
FK506	0.0001			96 (2)			89			
	0.001			29*	74 (2)	95 (2)	22*	80 (2)	78	
	0.01	-0.7 $\pm$ 0.5 (7)	8	11*	40*	82*	8*	69*	99	114*
H 186/86	0.1		87 (5)	95 (5)			82*			
	1	1.2 $\pm$ 0.4 (5)	27*	71*	100 (5)	104 (5)	107	99 (5)	88	98 (5)
	10	0.6 $\pm$ 0.3 (7)	11*	14*	139*	96	94	107	118*	143*
Metofenazate	0.69					102 (2)				
	6.9	0.6 $\pm$ 0.5 (7)	88	66*	97	115*	64*	114*	85	77*
	69	2.0 $\pm$ 0.5 (7)	20*	15*	84*	106	17*	123*	69*	50*
R 59 022	6.9	2.5 $\pm$ 1.3 (6)	62*	97	103	96	126*	129*	84	111
	69	3.7 $\pm$ 1.0 (6)	138*	82	120*	117*	122*	188*	84	118*
Ro 22-4839	0.69		69 (5)	80						110 (3)
	6.9	2.8 $\pm$ 1.4 (6-8)	31*	43*	102	95	82	105	78 (3)	122*
	69	2.8 $\pm$ 1.1 (6)	40*	13*	88	92	32*	113	49*	73*

The Table gives for each concentration of the indicated modulator the mean percentage release (mean recorded net release in the presence of the drug divided by mean expected release times 100). Unless otherwise stated, each figure is derived from at least six independent experiments. Concentrations of stimuli are those specified in legend to Fig. 1. Significant inhibitions or enhancements of release are indicated by an asterisk ($p < 0.05$ Wilcoxon's signed rank test on paired observations).

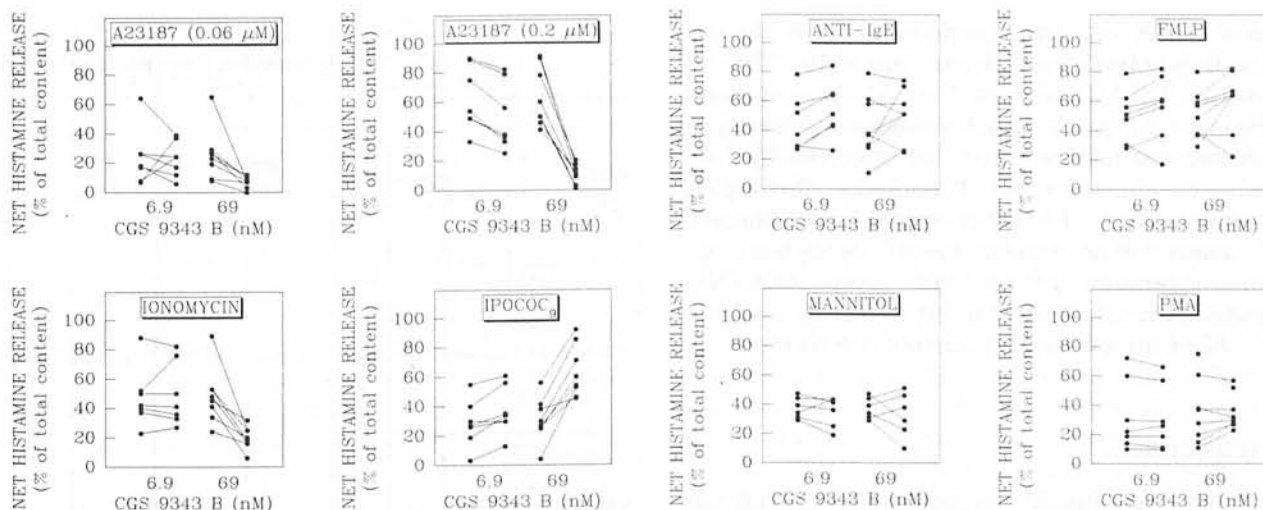


Fig. 2. Effects of CGS 9343B on leukocyte histamine release triggered by the indicated secretagogues. The figure gives for each concentration of the modulator the expected release (E – left point in each pair) and the actually recorded net release (R – right point in each pair), as defined in the experimental section, for cells exposed to the drug approximately 5 min before the secretagogue. Final concentrations of the secretagogues are as follows: A23187 0.06 μ M or 0.2 μ M as specified; anti-IgE 1:10000 dilution of antiserum; FMLP 1 μ M; ionomycin 0.15 μ M; IpOCOC₉ 206 μ M; mannitol 0.75 M and PKC 160 nM.

the expected release. The figures obtained in this way are given in Table 1.

As can be seen, CGS 9343B did not *per se* trigger histamine release at either 6.9 μ M or 69 μ M. In its presence, IpOCOC₉-induced histamine release, and to a lower degree that triggered by FMLP, was augmented whereas responses induced by the high concentration of A23187 or by ionomycin were reduced (Fig. 2, Table 1). The low concentration of CGS 9343B also enhanced anti-IgE-induced release, whereas the high concentration inconsistently affected responses to this secretagogue. A similar dichotomy of effects was observed at responses triggered by the low concentration of A23187 or by PMA (Fig. 2). We do not consider these apparently inconsistent effects as being due to unsatisfactory experimental conditions since they were obtained in experimental setups in which the compound simultaneously affected responses induced by the other employed secretagogues in a consistent way.

Table 1 also gives the mean degree of recorded release as percentage of the corresponding mean degree of expected histamine release for each concentration of other employed modulators. These figures show the following:

Effects of calmidazolium. Calmidazolium was found to trigger histamine release at 21 μ M (results not shown); it was therefore examined for modulatory effects at 2.1 μ M and 6.9 μ M. The compound affected histamine release triggered by the different secretagogues much as CGS 9343B did. However, calmidazolium failed to influence anti-IgE- and FMLP-induced release and reduced, but did not abolish, ionophore-triggered release.

Effects of felodipine. Felodipine at 10 μ M inhibited the responses triggered by both examined calcium ionophores and by FMLP but enhanced the responses triggered by IpOCOC₉.

Effects of H 186/86. The compound H 186/86, a t-butyl analogue of felodipine which binds to calmidazolium but lacks antagonistic activity, effectively reduced histamine release triggered by A23187 but did not affect that induced by ionomycin, IpOCOC₉ or FMLP. This is the case even if H 186/86 is examined at 100 μ M (results not shown). The compound augmented anti-IgE- and PMA-triggered responses.

Effects of metofenazate. Metofenazate affected basophil responses similar to CGS 9343B with the exception that the compound reduced PMA- but did not affect anti-IgE-triggered release.

Effects of Ro 22-4839. The effects recorded with Ro 22-4839 differed from those of CGS 9343B in that the enhancement of IpOCOC₉- and FMLP-triggered release seen with the latter compound was not seen with Ro 22-4839. However, it consistently blocked ionophore-induced histamine release and slightly reduced PMA-induced responses.

Effects of R 59 022. The compound R 59 022 markedly enhanced histamine release triggered by IpOCOC₉ and moderately augmented that induced by ionomycin, anti-IgE, FMLP or PMA but not that triggered by A23187.

Effects of FK506. The compound FK506 did not *per se* trigger histamine release even at 10^{-6} M (not shown). 10^{-8} M and even 10^{-9} M the compound completely blocked calcium ionophore-triggered release and partially reduced that triggered by IpOCOC₉ and anti-IgE without markedly affecting the release induced by FMLP or by PMA. Qualitatively and quantitatively similar results throughout (i.e. partial inhibition of anti-IgE- and IpOCOC₉-triggered release) were recorded also at 10^{-7} M and 10^{-6} M of the compound (results not shown).

Discussion

The salient findings of the present work, schematically depicted in Fig. 3, can be summarized as follows:

(i) Human leukocyte histamine release triggered by calcium ionophores is blocked by calmodulin antagonists implying a) that these agents do express inhibitory activity under the present conditions provided that an adequate stimulus is employed, and b) that calmodulin may constitute a link in a signal transduction pathway triggered by calcium ionophores.

(ii) None of the examined calmodulin antagonists affect histamine release induced by the different stimuli in the same way, implying that they display a partial functional selectivity. The effects recorded with these compounds also differ from those of two other purported calmodulin antagonists, W-7 (27) and polymyxin B (15), in that W-7 partially reduces PMA- and anti-IgE-triggered histamine release, whereas polymyxin B, if anything, enhances ionophore-induced responses (Bergstrand et al., submitted).

(iii) R 59 022 augments responses triggered by ionomycin but not those induced by A23187, whereas H 186/86 blocks A23187- but not ionomycin-triggered responses, implying partly different modes of action of these two purported calcium ionophores.

(iv) FK506 effectively blocks calcium ionophore-triggered responses and partly reduces those induced by anti-IgE and IpOCOC₉, implying that a PPI activity may be involved in these processes.

Although the findings presented are phenomenological in nature and do not unequivocally clarify signal transduction pathways in human basophil histamine release, they deserve the following comments:

Selectivity of employed modulators

The compounds employed as calmodulin antagonists reportedly possess some selectivity. CGS 9343B blocks calmodulin-stimulated cAMP phosphodies-

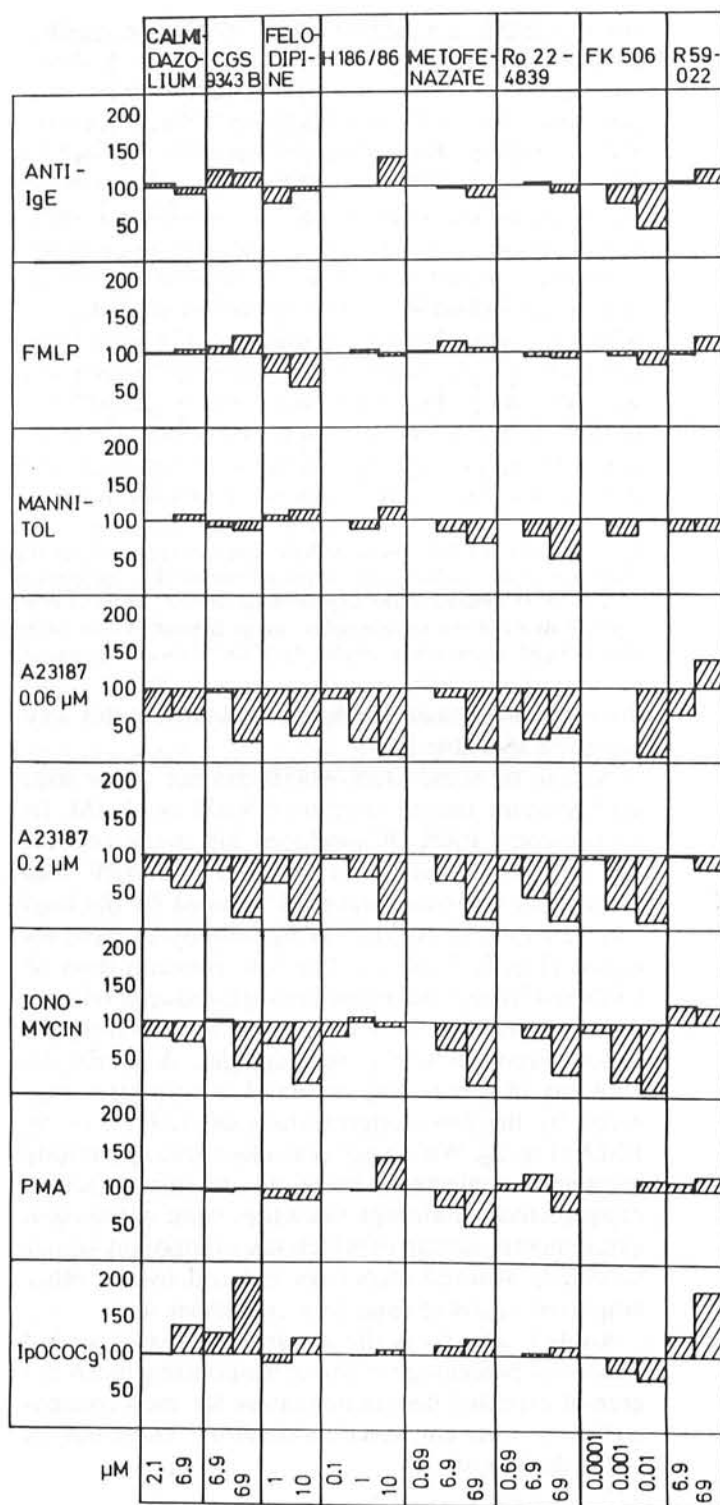


Fig. 3. Schematic summary of recorded effects of the examined modulators. The figure depicts the mean degree of histamine release triggered by each secretagogue in the presence of the indicated concentration of modulator as percentage of mean degree of release in its absence.

terase with an IC_{50} value of $3.3 \mu M$ and calmodulin-dependent protein kinase activity at less than $50 \mu M$ without affecting PKC even at $100 \mu M$ (16, 34). Ro

22-4839 is a potent antagonist of calmodulin toward myosin light chain kinase (IC_{50} value $3.1 \mu M$); it binds to the hydrophobic region of calmodulin at a site different from that binding trifluoperazine and W-7. The compound does not affect cAMP-dependent protein kinase or PKC (32). Metofenazate is a more selective calmodulin antagonist than trifluoperazine expressing a low degree of interaction with troponin C (45); whether the compound affects PKC activity is not clear. However, the activity of neither of two PMN leukocyte cytosolic PKCs is affected by the compound (Möller & Bergstrand, unpublished). Calmidazolium, another potent calmodulin antagonist, also reportedly affects other calcium-binding proteins such as troponin C (13) and calpain (50). Felodipine is a potent dihydropyridine type calcium channel blocker but also a calmodulin antagonist; its t-butyl analogue H 186/86, despite binding strongly to calmodulin, lacks functional calmodulin antagonism and pharmacological activity (46). Felodipine also activates platelet PKC (IC_{50} approx. $10 \mu M$ (42)). Moreover, R 59 022, an inhibitor of DAG kinase activity, causes an elevated level of intracellular DAG in platelets and neutrophils (11, 20) but the compound also reduces activities of phospholipase A_2 and DAG lipase (18, 26) and potentiates human neutrophil oxidative burst by raising intracellular levels of calcium (33). FK506 is a more potent immunosuppressive agent than cyclosporine A which most likely acts on the PPI named fujiphilin or FKBP (5, 12, 14, 17, 19, 23, 39, 43).

Effects of calmodulin antagonists

All examined calmodulin antagonists blocked ionophore-induced histamine release implying that calmodulin is an important link in this process. With this background, the lack of effect of calmidazolium, felodipine and CGS 9343B and the low degree of inhibition recorded with Ro 22-4839 on PMA-triggered basophil histamine release suggest that calmodulin plays no major role in the latter response. This view conforms to observations that calcium is not obligate for PMA-induced histamine release (2, 38, 48) and to the general consensus that phorbol esters trigger cellular responses without increasing intracellular calcium levels. A corollary of this view is that the reduction of PMA-triggered histamine release recorded with metofenazate is not due to calmodulin antagonism but to another mechanism. The related patterns of inhibition of histamine release seen with metofenazate (present work) and TMB-8 and W-7 (Bergstrand et al., submitted) suggest a common mode of action for these compounds; interference with a PKC-like activity is one possibility.

Most calmodulin antagonists did not influence anti-IgE-, FMLP- or hyperosmolar mannitol-induced responses. Notwithstanding the inhibitory effects of W-7 (Bergstrand et al., submitted), these results lend little support to the previous suggestion (27) that calmodulin plays an important *general* role in signal transduction pathway(s) in the basophil.

Felodipine was reported to partially reduce anti-IgE-induced basophil histamine release (4). We found felodipine to be an effective inhibitor also of calcium ionophore-triggered basophil histamine release. Conceivably, this may be due either to an interaction between the compound and component(s) of the putative calcium channel activated by the ionophore or to calmodulin antagonism. The finding that the t-butyl analogue of felodipine, H 186/86, which lacks functional calmodulin antagonism and pharmacological activity (46), blocks A23187-triggered responses but not ionomycin-induced ones indicates a) that felodipine expresses at least two modes of action in this system of which only one is mimicked by H 186/86, and b) that these two ionophores act in a slightly different manner.

Effects of R 59 022

R 59 022 slightly augments the leukocyte response to ionomycin. This effect is possibly due to a R 59 022-induced increase in DAG which in turn could synergize with Ca^{2+} introduced into the cell by the ionophore to activate a PKC. To explain why a similar enhancement by R 59 022 of the A23187-triggered release is not recorded, one may speculate that the latter also involves a phospholipase A_2 activity sensitive to R 59 022 (26) – this may be the reason why histamine release induced by the low concentration of A23187 is reduced by $6.9 \mu M$ R 59 022 (Table 1) – and that this effect counteracts the enhancement that would follow a R 59 022-mediated DAG kinase inhibition. An analogous situation is described in the platelet system (17).

$IpOCOC_9$ enhances the main PKC activity of neutrophils by replacing either the phorbol ester or calcium and phospholipid and, accordingly, synergizes with PMA as well as with A23187 in the leukocyte histamine release system (3, 35). The enhancement of $IpOCOC_9$ -induced release seen with R 59 022 therefore also possibly depends on a R 59 022-induced increase of DAG which synergizes with an $IpOCOC_9$ -mediated functional mimicking of the calcium limb in augmenting a PKC activity.

Effects of FK506

The efficacy of FK506 as an inhibitor of calcium ionophore-triggered basophil histamine release and its slightly lower effectiveness on anti-IgE-triggered

responses with no action on FMLP- or PMA-induced responses completely conform to characteristics recently reported for cyclosporine A and FK506 in this and a similar system (6, 12, 17). These results suggest that FK506 in the leukocyte blocks a pathway which requires (extracellular) calcium (23). It is conceivable that the target of FK506 in the basophil is fujiphilin/FKBP (17). The partial inhibitory effect of FK506 (and also of cyclosporine A at 10^{-6} M; Bergstrand & Lundquist, unpublished results) on IpOCOC₉-triggered release contrasts with that of calmodulin antagonists; FK506 is one of a few agents capable of reducing histamine release triggered by IpOCOC₉. By inference, this suggests that IpOCOC₉ induces histamine release through a pathway which involves a calcium-sensitive, calmodulin-independent step shared by the release process triggered by anti-IgE.

We conclude from these studies on effects of calmodulin antagonist that the process of leukocyte histamine release involves calmodulin as an obligate link when it is triggered by a calcium ionophore but not when induced by anti-IgE, FMLP, hyperosmolar mannitol or PMA. On the contrary, a calmodulin-dependent mechanism may exert a negative influence on the release triggered by IpOCOC₉ since many calmodulin antagonists augment such a response. The PPI inhibitor FK506 also blocks ionophore-induced histamine release but in other aspects its action differs from that of calmodulin antagonists implying that FK506 does not act via calmodulin in this system. Finally, the effects of the DAG kinase inhibitor R 59 022 differ from those of calmodulin antagonists in that R 59 022 does not inhibit A23187-induced leukocyte histamine release.

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