Histamine-stimulated and GTP-binding Proteins-mediated Phospholipase A2 Activation in Rabbit Platelets*

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Histamine is known to be a mediator of inflammation. In order to understand the role of histamine in platelets, we have examined the effects of histamine on arachidonic acid (AA) release, cAMP accumulation, and serotonin secretion. Incubation of rabbit (and human) platelets with histamine resulted in rapid increase of [3H]AA release from the platelets prelabeled with [3H]AA. The effect of histamine was blocked by the addition of H1 receptor antagonist mepyramine. Histamine did not substantially affect the cAMP content and inositol trisphosphate production. Histamine-stimulated AA release was not observed in digitonin-permeabilized platelets, whereas histamine acted synergistically with GTP or GTP analog, guanosine 5'-[3-O-thio]triphosphate. Histamine-stimulated, and GTP analog-dependent AA release was inhibited by guanosine 5'-[2-O-thio]diphosphate. The effects of three receptor stimulants, thrombin, norepinephrine, and histamine were both diminished by 1 μg/ml of pertussis toxin treatment and by the antiserum against GTP-binding proteins (G proteins) treatment. However, the antiserum against βγ subunits of G proteins inhibited the histamine effect, not thrombin effect. 4β-Phorbol 12-myristate 13-acetate (PMA) treatment enhanced histamine-stimulated AA release and serotonin secretion but inhibited thrombin-stimulated reactions. The effect of PMA was dose dependent and was due to enhancement of histamine receptors and G proteins. The results show the existence of H1 histamine receptors which couple phospholipase A2 activation via pertussis toxin-sensitive G proteins. Histamine actions differ in sensitivities to anti βγ antiserum treatment and PMA treatment from thrombin actions.

There are many lines of evidence suggesting that histamine might act as neurotransmitter in the central nervous system. Additionally, histamine is established to be a chemical mediator of allergies and inflammation (Schwartz et al., 1986a, 1986b). Histamine action is believed to be mediated via cell surface receptors, which are classified to three distinct subtypes, H1, H2, and H3 receptors, by pharmacological studies. The histamine actions produced by H1 receptors including bronchoconstriction, vasconstriction, increased capillary permeability are blocked by mepyramine (Schwartz et al., 1986a, 1986b). Recently, stimulation of H1 receptors can induce breakdown of inositol phospholipids in cerebral cortical slices (Hollingsworth and Daly, 1985), hippocampus (Baudry et al., 1986), A431 human epidermoid carcinoma cells (Hepler et al., 1987). The histamine actions mediated by H2 receptors including cardiac dysfunction and gastric acid secretion are blocked by cimetidine which was developed by Block et al. (1972). Stimulation of H2 receptors can induce cAMP accumulation in a variety of tissues including human platelets (Klysen et al., 1980). H3 receptors appear to be presynaptic autoreceptors, only recently discovered in brain, which control histamine synthesis and release (Arrang et al., 1985). However, the mechanism of signal transduction via H3 receptors has never been known.

Several investigations suggest that histamine modulates the human platelet functions via the histamine receptors (Klysen et al., 1980; Norn et al., 1982). They reported that stimulation of both H1 and H2 receptors reduce the serotonin secretion by thrombin. And Giap et al. (1986) reported the action of histamine on serotonin uptake by human platelets. They suggest the regulatory role of histamine and the involvement of a new subclass of H2 receptors mediating serotonin uptake.

There is increasing evidence that the breakdown products of inositol phospholipids by phospholipase C, IP3, and diacylglycerol are important intracellular messengers to play a key role in stimulus-secretion coupling in many tissues including platelets (Berridge, 1984; Nishizuka, 1986). In addition, AA released by phospholipase A2 or its metabolites had functional roles in platelets (Authi et al., 1986; Watson et al., 1986; Kajiyama et al., 1986). In the present study we have investigated the effect of histamine on rabbit platelet functions such as IP3 formation, AA release, and serotonin secretion. Here we observed that the addition of histamine stimulated AA release from rabbit (and human) platelets via H1 receptors and enhanced PMA-stimulated serotonin secretion. The activation of phospholipase A2 by histamine appears to be mediated by IAP-sensitive G proteins, like other receptor stimulants, thrombin or norepinephrine. However, the mechanism of phospholipase A2 activation by histamine appears to be different from that of thrombin, since the sensitivities to the antiserum against βγ subunits of G proteins and to PMA were not equally observed.

EXPERIMENTAL PROCEDURES

Materials—Histamine HCl, thrombin, norepinephrine, GTP, PGE2, and PMA were purchased from Sigma. GTPTs and GDPβS

The abbreviations used are: IP3, inositol trisphosphate; AA, arachidonic acid; G proteins, GTP-binding proteins; Gα, a GTP-binding protein of adenylyl cyclase that mediates inhibition; Gβγ, a GTP-binding protein of unknown function; GDI/βγ, guanosine 5'-[2-O-thio]diphosphate; GTPTs, guanosine 5'-[3-O-thio]triphosphate; IAP, islet-activating protein (pertussis toxin); Heps, 4-[2-hydroxyethyl]-1-piperazineethanesulfonate; PGE2, prostaglandin E2; PMA, 4-phorbol 12-myristate 13-acetate; EGTA, ethylenebis(oxyethylene nitrilo) tetraacetic acid.

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were from Boehringer-Mannheim. [5,6,8,9,11,12,14,15-3H]AA (80-140 Ci/mmol), 5-hydroxytryptamine (serotonin) (20-40 Ci/mmol), and myo-[2-3H]inositol (10-20 Ci/mmol) were purchased from Amer sham Corp. or Du Pont-New England Nuclear. Cimetidine and me- pyramine fumarate were gifts from Smith, Kline, Fujisawa Corp., and Fujisawa-yakuhin Corp. Indomethacin was a gift from Redary Corp. IAP, purified from 2-day culture supernatant of Bordetella pertussis, was a generous gift from Kaken-seiyaku (Kyoto, Japan). Reagents for radioimmunoassay of cAMP were generously donated by Yamasa Shoyu, Co., Ltd. (Chiba, Japan). The sources of other materials were those described in previous papers (Murayama and Uii, 1985, 1987a, 1987b; Kajiyama et al., 1989; Murayama et al., 1990).

Purified G proteins and their βγ subunits were prepared by the methods of Kitamura et al. (1987). Anti-Gi/Gs antiserum and anti-βγ antiserum were prepared by Dr. Y. Kitamura in this laboratory according to the previous report (Kitamura et al., 1989). Anti-Gi/Gα antiserum reacted with β subunits of G proteins (data not shown, but see Kitamura et al., 1989).

Isolation of Rabbit, Human Platelets, and Prior Radiolabeling with 3H[AA] or [H]Serotonin and Pretreatment with PMA—Rabbit and human platelets were isolated on the day of experiment and labeled with [3H]AA or [3H]serotonin as described in the previous papers (Kajiyama et al., 1989; Murayama et al., 1990). Briefly, platelet-rich plasma was obtained by centrifugation of the blood at 150 × g for 10 min at 20°C, and platelet-rich suspension was obtained by gel filtration using Sepharose-2B. Platelets were separated by centrifugation at 1500 × g for 10 min at 4°C and washed twice with a modified Tyrode Hepes buffer (134 mM NaCl, 12 mM NaHCO3, 2.9 mM KCl, 0.34 mM Na2HPO4, 1 mM MgCl2, 10 mM Hepes, 5 mM glucose, 0.3% bovine serum albumin, pH 7.4). Platelets were incubated for 2 h at 37°C with the same Tyrode buffer containing [3H]AA (5 μCi/ml) or [3H]serotonin (5 μCi/ml) in the presence of 10 μM indomethacin. The labeled platelets were washed twice and finally suspended with the Tyrode buffer. In some experiments, PMA or the solvent dimethyl sulfoxide (maximal final concentration, 0.2%) were added to the labeled platelets, and the incubation was continued for 10 min at 37°C. Thereafter, PMA-treated platelets were precipitated by the centrifugation.

Digitonin, IAP, and Anti-G Proteins Antiserum Treatment—In several experiments, the labeled platelet suspension was incubated with 10-15 μM digitonin for 7 min at 37°C. In Fig. 4, the incubation buffer was further supplemented with 1 μg/ml of the preactivated IAP, 100 μM NAD, 1 mM ATP, 10 mM thymidine, 10 mM dithiothreitol for ADP-ribosylation of membrane proteins. This digitonin treatment was terminated by dilution with 10 ml of the Tyrode buffer, and the permeabilized platelets were sedimented by centrifugation at 1500 × g for 10 min at 10°C. In Table III, the labeled and permeabilized platelets were incubated for 20 min at 30°C with 20 μl/ml of anti-Gi/Gα antiserum or anti-βγ antiserum.

[3H]AA Release and [H]Serotonin Secretion in Response to Stimuli—The intact or permeabilized platelets were incubated for 7 min to assay for [3H]AA release and for 1 min to assay for [3H]serotonin secretion at 37°C with 0.1 mM CaCl2 and further additions shown in the tables and figures. In Figs. 2, 3, and 9, these platelets were first incubated with histamine receptor antagonists for 5 min. The reaction was terminated by addition of 1 ml of the ice-cold Tyrode buffer containing 2 mM EGTA and 5 mM EDTA followed by a 2-min centrifugation at 8000 × g at 4°C. The 3H content of the supernatant was estimated by the liquid scintillation spectrometer.

Cyclic AMP Responses of Platelets—The intact platelets were suspended in the Tyrode buffer. Platelet suspensions were incubated for 5 min at 37°C in the presence of 0.5 mM 3-isobutyl-1-methylxan-thine. Further additions are shown in the legend to Table II. Incubation was terminated by acidification with HCl to final 0.1 N and by boiling for 2 min. The cellular cAMP quantitatively transferred to the supernatant was determined by a sensitive radioimmunooassay method (Kajiyama et al., 1977).

[3H]IP3, Formations of Platelets—Intact platelets were suspended in the Tyrode buffer and incubated for 2 h at 37°C in the presence of [3H]inositol (25 μCi/ml) in the presence of 10 μM indomethacin. The labeled platelets were washed twice with the buffer and incubated for 15 s at 37°C with additions as shown in Table I. The incubation was termina ted by addition of 10% trichloroacetic acid, followed by extraction with five washes of 5 volumes of water-saturated diethyl ether. The aqueous phase was applied to Dowex AG 1-X8 columns for separation of IP3 as described elsewhere (Berridge, 1984; Kajiyama et al., 1989).

RESULTS

H1 Histamine Receptor-stimulated AA Release—Fig. 1 shows that the radioactivities were increased in the medium when [3H]AA labeled rabbit platelets were incubated. The rate of release was constant for 10 min in the vehicle-stimulated platelets. The rate of release was enhanced upon the addition of 100 μM histamine. The enhancement was dependent on the concentration of histamine, the half-maximal increase with approximately 2-5 μM (Fig. 1B). When platelets were preincubated with the indicated concentrations of H1 receptor antagonist mepyramine, the stimulatory effect of histamine on [3H]AA release was attenuated in a dose-dependent manner (Fig. 2). No effect was observed by cimetidine. H2-selective antagonists.

Table I shows that thrombin provoked rapid formation of [3H]IP3 when added to rabbit platelets within 15 s as previously shown (Kajiyama et al., 1989). Histamine had no effect on IP3 formation. This finding suggests that histamine-stimulated AA release is mainly due to activation of phospholipase A2 but not to activation of phospholipase C. Klysner et al. (1989) reported that histamine caused an accumulation of cAMP via H2 receptors in human platelets. We examined the effect of histamine on cAMP content (Table II). The addition of 10 μM PGE2 stimulated cAMP accumulation about 80-fold in rabbit platelets. On the contrary histamine stimulated
Different Effects of PMA on Thrombin and Histamine Actions

TABLE I
Effects of thrombin and histamine on IP₃ formation

The [³H]inositol-labeled platelets were incubated with stimulants for 15 s to measure the formation of IP₃, as described under "Experimental Procedures." The concentrations of additions were thrombin, 1 unit/ml; histamine, 100 µM. The data are the means ± S.E. from three to four separate experiments.

| Additions | [³H]IP₃ formation (dpm/10⁶ cells) |
|-----------|----------------------------------|
| None      | 51 ± 8                           |
| Thrombin  | 429 ± 8*                         |
| Histamine | 59 ± 11                          |

* Effect of thrombin was significant (p < 0.01).

TABLE II
Cyclic AMP accumulation by histamine and PGE₁

Rabbit platelets were incubated for 10 min with the indicated additions and then assayed for cAMP accumulation as described under "Experimental Procedures." The concentrations of additions are histamine, 100 µM; mepyramine, 10 µM; cimetidine, 10 µM; PGE₁, 10 µM.

| Additions | cAMP accumulation (pmol/10⁶ cells) |
|-----------|-----------------------------------|
| None      | 0.550 ± 0.028                     |
| Histamine | 0.679 ± 0.024                     |
| +Mepyramine | 0.707 ± 0.061*                    |
| +Cimetidine | 0.565 ± 0.021                     |
| PGE₁      | 0.414 ± 0.20                      |
| +Histamine | 0.443 ± 0.20                      |

* Effect of histamine was significant (p < 0.01).

Fig. 3. Effect of histamine on [³H]AA release from human platelets. The [³H]AA-labeled human platelets were first incubated for 5 min with 10 µM mepyramine (○), cimetidine (▲), or the vehicle (△) and further incubated for 7 min with various concentrations of histamine. The data are the means ± S.E. from three separate experiments.

cAMP accumulation about 1.2-fold. This small effect of histamine was blocked by the addition of 10 µM cimetidine, not mepyramine. Histamine did not inhibit cAMP accumulation even in the presence of PGE₁. The addition of 10 µM PGE₁ did not stimulate [³H]AA release (data not shown). These data suggest that the observed effect of histamine on AA release is probably mediated by H₂ subtype of histamine receptors and not by H₁ subtype and adenylyl cyclase systems.

Histamine-stimulated [³H]AA release was also observed in human platelets (Fig. 3). The half-maximal increase was about 0.2 µM, and the effect of histamine was blocked by the addition of 10 µM mepyramine. These results are in accordance with the previous report suggesting the existence of H₁ receptors in human platelets (Norn et al., 1982).

G Proteins Interactions with Histamine Receptors and Phospholipase A₂—The responses to histamine or other receptor stimulants, such as thrombin and norepinephrine, were examined using permeabilized rabbit platelets (Figs. 4 and 5). Histamine-stimulated [³H]AA release was not observed in the permeabilized platelets, in contrast with intact platelets (Fig. 4C). When the reaction mixture was supplemented with GTP, [³H]AA release was markedly stimulated by histamine. As shown in our previous reports (Kajiyama et al., 1989; Murayama et al., 1990), thrombin- and norepinephrine-stimulated AA release were dependent on the coexistence of GTP (Fig. 4, A and B). Without receptor stimulation, hydrolysis-resistant GTP analog GTP₇S evoked the release of [³H]AA in the permeabilized platelets (Fig. 5A). This GTP₇S-induced AA release was enhanced by histamine. As shown in Fig. 5B, both responses by 10 µM GTP₇S in the absence or presence of 100 µM histamine were reduced by the stable GDP analog GDP₇S. GDP₇S inhibited those responses in a dose-dependent manner, and the half-maximal inhibition required about 50 nM, respectively.

Histamine-stimulated AA Release Prevented by IAP and Anti-G Proteins Antiserum Treatment—Since the above data suggested the involvement of G proteins in AA release by

Fig. 4. GTP-dependent stimulation of AA release by receptor stimulants and its inhibition by IAP treatment. The [³H]AA-labeled platelets were first incubated with 16 µM digitonin plus 100 µM NAD in the absence (○) or presence (▲) of 1 µg/ml of preactivated IAP and then assayed for [³H]AA release as described under "Experimental Procedures." The assay mixture was supplemented with (▲) or without (○) 10 µM GTP and indicated concentrations of thrombin (panel A), norepinephrine (panel B) or histamine (panel C). The left points of each panel indicate the basal [³H]AA releases without receptor stimulants. The data are mean of duplicate experiments.

Fig. 5. Antagonism of GTP₇S-stimulated AA release by GDP₇S in the absence or presence of histamine. Panel A, [³H] AA-labeled and permeabilized platelets were incubated with increasing concentrations of GTP₇S in the absence (●) or presence (▲) of 100 µM histamine. Panel B, the platelets were incubated with 100 µM GTP₇S plus indicated concentrations of GDP₇S in the absence (●) or presence (▲) of 100 µM histamine. The data are the mean of duplicate separate experiments.
histamine, the effect of IAP was investigated. IAP, which catalyzes the ADP-ribosylation of certain G proteins such as G\text{a} and G\text{b}, is used as a probe to study mechanisms involved in receptor-mediated signaling transduction in a variety of cell types (see for review, Ui, 1984; Murayama and Ui, 1985, 1987b; Nomura et al., 1985). In phospholipase A\textsubscript{2} assay system using permeabilized platelets, IAP treatment blocked thrombin-, norepinephrine-, and histamine-stimulated [\textsuperscript{3}H]AA releases (Fig. 4). In Table III, the effects of two kinds of anti-G proteins antisera were examined. Pretreatment with anti-G\text{a}/G\text{b} antisera, which reacted both \(\alpha\) and \(\beta\) subunits of G\text{a} and G\text{b}, blocked thrombin- and histamine-stimulated AA release 82 and 92\%, respectively. These data suggest that the involvement of IAP-sensitive G\text{a} proteins in receptors-mediated activation of phospholipase A\textsubscript{2} including histamine receptors. On the other hand, pretreatment with anti-\(\beta\gamma\) antisera, which mainly reacted \(\beta\gamma\) subunits of G\text{b} proteins, blocked the effect of histamine, but not the effect of thrombin. This finding suggests that the participation of G proteins in phospholipase A\textsubscript{2} activity between thrombin and histamine receptors is not the same manner, in view of the role of \(\beta\gamma\) subunits, while the exact roles of each subunit (\(\alpha\) and \(\beta\gamma\)) of G proteins is unknown.

Enhancement of Histamine-, Not Thrombin- and Norepinephrine-, stimulated AA Release by PMA Pretreatment—Phorbol esters such as PMA cause platelet activation at high doses (Rink et al., 1989). However, several findings indicate that the activation of protein kinase C could induce feedback inhibition of doses (Rink et al., 1989). Pretreatment with anti-G\text{a}/G\text{b} antisera, which reacted both \(\alpha\) and \(\beta\) subunits of G\text{a} and G\text{b} blocked thrombin- and histamine-stimulated AA release and diminution of the thrombin-stimulated serotonin secretion at high doses, and diminution of the thrombin-stimulated serotonin secretion at low doses (Murayama et al., 1990). Thrombin addition of 10 \(\mu\)M 1-(5-isouquinilinylsulfonyl)-2-methylpiperazone (H-7), a protein kinase C inhibitor (data not shown).

The stimulatory effect of PMA on histamine-stimulated [\textsuperscript{3}H]AA release was observed when using permeabilized platelets (Fig. 8). The maximal response by histamine plus GTP- or GTP\textsubscript{S}-stimulated AA release in permeabilized platelets was markedly enhanced, and the affinity for histamine was increased by 10 \(\mu\)M PMA pretreatment. As shown in Fig. 8C, GTP\textsubscript{S}-stimulated reaction without receptor stimuli was not reduced in the PMA-treated platelets. The ED\textsubscript{so} values for GTP\textsubscript{S} in histamine-stimulated AA release were 1 \(\mu\)M and 25 \(\mu\)M in control and PMA-treated platelets, respectively. These results suggest that the coupling between the histamine receptors and the G proteins leading to AA release, not between the G proteins and phospholipase A\textsubscript{2}, is positively modified by protein kinase C activation.

**Table III**

| Additions          | [\textsuperscript{3}H]AA released |
|-------------------|----------------------------------|
|                   | Control serum | Anti-G\textsubscript{a}/G\textsubscript{b} antisera | Anti-\(\beta\gamma\) antisera |
| None              | 100           | 102 ± 1.2 | 97.8 ± 6.5 |
| Thrombin          | 100 (100 ± 10)| 18.4 ± 0.5\textsuperscript{a} | 101 ± 2.3 |
| Histamine + GTP   | 100 (1100 ± 12)| 8.2 ± 6.1\textsuperscript{a} | 25.9 ± 11.8\textsuperscript{a} |

\(
\textsuperscript{a}\) Effect of the antisera was significant (\(p < 0.01\)).

**Fig. 6. Different effects of PMA treatment on thrombin-, norepinephrine-, and histamine-stimulated AA releases.** The [\textsuperscript{3}H]AA-labeled platelets were pre-exposed for 10 min to 10 \(\mu\)M PMA (●) or the vehicle (○) as described under “Experimental Procedures.” The washed platelets were incubated for 7 min with indicated concentrations of thrombin (panel A), norepinephrine (panel B), and histamine (panel C). The left points of each panels indicate the basal [\textsuperscript{3}H]AA releases without receptor stimulants. The data are the mean of duplicate separate experiments.

**Fig. 7. Concentration dependence of PMA enhancement on histamine-stimulated AA release.** The [\textsuperscript{3}H]AA-labeled platelets were pre-exposed to the indicated concentrations of PMA for 10 min, and the washed platelets were incubated for 7 min with 100 \(\mu\)M histamine. The effect of PMA was plotted as a percentage of 100 \(\mu\)M histamine-stimulated [\textsuperscript{3}H]AA release from control (without PMA treatment) platelets, which was 358 ± 37 dpm/10\textsuperscript{6} cells.

addition of 10 \(\mu\)M 1-(5-isouquinilinylsulfonyl)-2-methylpiperazone (H-7), a protein kinase C inhibitor (data not shown).

The stimulatory effect of PMA on histamine-stimulated [\textsuperscript{3}H]AA release was observed when using permeabilized platelets (Fig. 8). The maximal response by histamine plus GTP- or GTP\textsubscript{S}-stimulated AA release in permeabilized platelets was markedly enhanced, and the affinity for histamine was increased by 10 \(\mu\)M PMA pretreatment. As shown in Fig. 8C, GTP\textsubscript{S}-stimulated reaction without receptor stimulants was not reduced in the PMA-treated platelets. The ED\textsubscript{so} values for GTP\textsubscript{S} in histamine-stimulated AA release were 1 \(\mu\)M and 25 \(\mu\)M in control and PMA-treated platelets, respectively. These results suggest that the coupling between the histamine receptors and the G proteins leading to AA release, not between the G proteins and phospholipase A\textsubscript{2}, is positively modified by protein kinase C activation.

**PMA-stimulated Serotonin Secretion Is Enhanced by Histamine Addition**—We previously reported that PMA treatment induced both phenomena, stimulation of serotonin secretion at high doses, and diminution of the thrombin-stimulated secretion at relatively low doses (Murayama et al., 1990). Fig. 9 shows that 100 nM PMA pretreatment produced a significant release of the radioactivity from [\textsuperscript{3}H] serotonin-loaded platelets in the Tyrode buffer containing 100 \(\mu\)M Ca\textsubscript{2}+.
Histamine did not change platelets (and human platelets) was inhibited by the addition of H1 antagonist, not histamine in the brain (Schwartz et al., 1986a, 1986b). The Hz subtype which is coupled to stimulation of adenylate cyclase (Murayama et al., 1989). We now measure the levels of IP3 in rabbit platelets (Table I). 3) The histamine-sensitive cAMP accumulation, which appears to be uniformly coupled to H1 receptors, was very small (Table II). Under the same experimental conditions, PGE2 induced a marked (90-fold) increase. Taken together these results show that histamine-stimulated AA release in rabbit platelets is mediated by H1 subtype of histamine receptors mainly. A possibility of the involvement of Hz subtype receptors is discussed below. 4) In permeabilized platelets, histamine stimulated AA release in the presence of GTP analogs (Figs. 4 and 5). Histamine-stimulated release in the presence of GTPyS was antagonized by GDPβS addition (Fig. 5B). 6) Not only thrombin action but also histamine action were reduced by pretreatment with IAP (Fig. 4) and anti-C3/C5 antisemum (Table III). In rabbit platelets, Ca2+ is an important factor to the activation of phospholipase A2 but is not the sole factor to the regulation. G proteins are involved in receptor-mediated activation of phospholipase A2 (Nakashima et al., 1987a, 1987b; Fuse and Tai, 1987; Kajiyama et al., 1989). These results suggest that IAP-sensitive G proteins are involved in both thrombin receptor- and H1 histamine receptor-mediated activation of phospholipase A2.

Specificity in Signal Transduction of Histamine—The results indicated herein show that the signal transduction pathways activated by histamine that lead to AA release can be distinguished from those utilized by thrombin or norepinephrine. First, the sensitivity for anti-βγ antisemum is different (Table III). Both thrombin- and histamine-stimulated AA release were inhibited by IAP treatment and by anti-C3/C5 antisemum treatment. Anti-βγ antisemum treatment, however, inhibited the histamine action not the thrombin action. The reasons for this difference are not clearly understood. The βγ subunits can modulate the function of several effectors (Neer and Clapham, 1988) including retinal phospholipase A2 (Jel-sema and Axelrod, 1987). Our results show that the role of subunit of G proteins may be different between thrombin receptors and histamine receptors.

Second, the sensitivity for PMA is different (Figs. 6 and 9). A brief exposure to PMA markedly inhibited thrombin-stimulated AA release (Fig. 6A) and serotonin secretion (data not shown, but see Murayama et al., 1990). The inhibitory effect could be due, at least in part, to uncoupling between receptors and G proteins by activation of protein kinase C (Murayama et al., 1990). In contrast, PMA treatment enhanced histamine-stimulated AA release and serotonin secretion. The present findings are somewhat analogous to the observations that PMA inhibits the inositol phosphates production by α1-adrenergic agonists on hepatocyte, but not those by vasopressin and angiotensin II (Cooper et al., 1985; Corvera et al., 1986). Similarly, activation of protein kinase C by PMA results in inhibition of vasopressin- and bombesin-stimulated increases of cytosolic Ca2+ concentration but leaves the platelet-derived growth factor-stimulated response (Lopes-Rivas et al., 1987). The reason for this selective sensitivity for one receptor but not another is not clear. One possible explanation for this effect is a better coupling between histamine receptors and G proteins (Fig. 8). Another possible explanation is the modification of receptor numbers. PMA is known to modulate several agonists binding to its receptors in many tissues (Motozaki et al., 1986; Bjorge and Kudlow, 1987; Davis and Meisner, 1987). In Swiss 3T3 fibroblasts PMA treatment induced to express more A2 adenosine receptors, not β-adrenergic receptors, enabling binding of the ligand and coupling to adenylate cyclase (Murayama et al., 1989). We now measure the adenosine binding for histamine receptors.

**DISCUSSION**

H1 Histamine Receptor, G Proteins, and Phospholipase A2 System in Rabbit Platelets—The results presented herein show, for the first time to our knowledge, that rabbit (and human) platelets have a pathway for H1 histamine receptors-mediated activation of phospholipase A2 and that PMA is a potent stimulator of this histamine-stimulated pathway. There are three types of histamine receptors, the H1 subtype which is coupled to breakdown of inositol phospholipids, the H2 subtype which is coupled to stimulation of adenylate cyclase, and the H3 subtype which is coupled to uptake of histamine in the brain (Schwartz et al., 1986a, 1986b). The following findings suggest that activation of phospholipase A2 is coupled to H1 histamine receptor by G proteins in rabbit platelets. 1) Histamine-stimulated [3H]AA or its metabolites release from [3H]AA-labeled rabbit platelets (and human platelets) was inhibited by the addition of H1 antagonist, not by H2 antagonist (Figs. 2 and 3). Histamine did not change serotonin secretion. The [3H]serotonin-loaded platelets were pre-exposed to 100 nM PMA (A), cimetidine (m), or the vehicle (O) for 10 min and further incubated for 1 min with the indicated concentrations of histamine. Other details are described under "Experimental Procedures." The data are the mean ± S.E. from three separate experiments. The effect of PMA was enhanced by histamine addition in a dose-dependent manner with half-maximal stimulation occurring at approximately 2 μM, while histamine, even if 1 mM used, had no effect on serotonin secretion. The effect of histamine was blocked by 10 μM mepyramine.
is known that AA and its metabolites such as thromboxanes elicit platelet aggregation and secretion. The addition of inhibitor of Na+/H+ exchange blocked both AA release and platelet secretion of dense granule contents in response to epinephrine and ADP (Sweatt et al., 1985). IP3-induced aggregation and serotonin secretion from permeabilized human platelets were diminished by the cytochrome oxidase inhibitor, indomethacin and aspirin (Authi et al., 1986; Watson et al., 1986). Thrombin-stimulated serotonin secretion was decreased by mepacrine, an inhibitor of phospholipase A2 (Kajiyama et al., 1989). These reports suggest that the AA release by phospholipase A2 activation has an important role in platelet function such as aggregation and serotonin secretion. Our results showing that histamine stimulated AA release and enhanced PMA-induced serotonin secretion (Fig. 9) are in accordance with previous reports.

Several investigators demonstrated a regulatory role of histamine on human platelet function via H1 and H2 receptors (Klysner et al., 1980; Norin et al., 1982). Our results also suggest that H1 histamine receptors can regulate platelet function such as AA release and serotonin secretion (Fig. 9). However, the existence and the role of H2 subtype of histamine receptors, which is coupled to uptake of histamine in the brain (Schwartz et al., 1986a, 1986b), is not declined. Gespach et al. (1986) reported the involvement of a subpopulation of H2 receptors on serotonin uptake to human platelets. Histamine molecules incorporated may be able to modulate ADP-ribosylation of G proteins coupled to phospholipase A2, since cellular ADP-ribosyltransferase is shown to be inhibited by histamine (Lee and Iglewski, 1984).

Recently, Saxena et al. (1989) reported that PMA increased histamine content in parallel with promotion of aggregation in human platelets. Their results showing the potentiation of PMA action by histamine is in accordance with our results that PMA promoted histamine-stimulated AA release and serotonin secretion (Fig. 9). However, they suggest that histamine is an intracellular messenger because inhibitors of histidine decarboxylase suppressed PMA-stimulated aggregation. In our preliminary experiments, histamine-stimulated AA release from intact or permeabilized platelets was not inhibited by a-methylhistidine, an inhibitor of histidine decarboxylase (data not shown). Further study is required to clarify the role of histamine on platelet functions.

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