Biochemical Pharmacology, Vol. 40, No. 8, pp. 1677–1681, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00
© 1990. Pergamon Press plc

COMMENTARY

HISTAMINE AS AN INTRACELLULAR MESSENGER

LORNE J. BRANDES,*†‡§ FRANK S. LABELLA,† GARY B. GLAVIN,† FRIXOS PARASKEVAS,*‡
SATYA P. SAXENA,‡ ARCHIBALD MCNICOL‡ and JON M. GERRARD‡‖

Departments of *Medicine, †Pharmacology and Therapeutics, and ‖Pediatrics, and the ‡Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, MB R3E OV9, Canada

Through the synthesis of *N,N*-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine·HCl (DPPE) [1], a potent ligand for the microsomal antiestrogen binding site (AEBS) to which tamoxifen (TAM) binds [2], we have identified the existence of a novel low (μm) affinity intracellular histamine receptor (designated H_{IC}), which may be associated with AEBS. As a result of binding to H_{IC}, histamine (a) mediates platelet aggregation, and (b) as postulated by Kahlson and Rosengren twenty-five years ago [3], is implicated in cell proliferation and other important physiological processes.

Histamine as a second messenger: development of the concept

Histamine and cell proliferation. In the 1960s, Kahlson proposed that intracellular histamine is an important mediator of cell growth [3]. He found the conversion of [^{14}C]histidine to [^{14}C]histamine to be increased significantly in rapidly growing tissues, and the urinary excretion of histamine to be elevated markedly in pregnancy and tumor implantation. He determined that such histamine does not derive from mast cells or gut flora, and hypothesized an intracellular origin. Although supported by much indirect evidence, Kahlson's hypothesis suffered at that time from lack of identification of a specific receptor at which intracellular histamine mediates a putative growth-promoting action, the unavailability of histamine receptor ligands or specific inhibitors of histamine formation with a recognized effect on proliferation, and conflicting data [4] on such a role for histamine from other laboratories.

In 1981, Watanabe *et al.* [5] first linked the tumor-promoting action of the phorbol ester, phorbol-12-myristate-13-acetate (PMA), to histamine production by showing that, in addition to activating protein kinase C (PKC), PMA also markedly increases histidine decarboxylase (HDC) and ornithine decarboxylase (ODC) activity within hours of application to mouse skin. ODC activity rises higher and appears earlier (within about 6 hr but declines rapidly by 12 hr), whereas HDC activity peaks at ten times basal level at 12 hr and remains elevated for 48–96 hr. At about the same time, Christensen and co-workers isolated a phorbol analogue from the root of *Thapsia garganica*. The derivative, aptly named thapsigargin, does *not* bind to PKC or induce ODC, but increases cytosolic calcium [6] and *is* a potent activator of HDC [7]. Moreover, thapsigargin is as effective a tumor promotor as PMA in the two-stage mouse skin carcinogenesis model [7], lending further credence to an important role for histamine in growth.

Synthesis of DPPE, a ligand for the microsomal antiestrogen binding site (AEBS), but not the estrogen receptor

A possible biological role for AEBS. In 1984, Brandes and Hermonat synthesized a *p*-diphenylmethane derivative, *N,N*-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine·HCl (DPPE) [1], in an attempt to find a ligand selective for the microsomal AEBS [2], but not the estrogen receptor (ER) [8]. The structure of DPPE resembles the prototype AEBS ligand, TAM (Fig. 1); however, presumably because of the absence of a stilbene linkage to a third phenyl ring, DPPE does not bind significantly to ER, while binding AEBS with relatively high affinity ($K_i \sim 60$–70 nM) [1]. Like TAM, DPPE at micromolar concentrations is antiproliferative/cytotoxic to various malignant cell lines *in vitro* [9] and inhibits uterine growth *in vivo* [10], suggesting a role for AEBS in proliferation.

Identification of a novel micromolar affinity histamine site which may be AEBS-associated. The structure of DPPE also resembles H_1 histamine antagonists such as phenyltoloxamine (the ortho-isomer of DPPE) and diphenhydramine (Fig. 1). Based on Kahlson's original hypothesis [3], the possibility was raised that antagonism of histamine action may represent a mechanism for the growth inhibitory effects of AEBS ligands such as TAM and DPPE. Binding studies in rat liver microsomes showed that H_1 antagonists, including phenothiazine derivatives and tricyclic antidepressants, compete with varying degrees of affinity for [^{3}H]TAM binding, whereas H_2 antagonists and other classes of drug agonists and antagonists do not [11]. On this basis, and because H_1 potency in functional assays does not correlate with that of AEBS binding in rat liver microsomes [12], it was hypothesized that DPPE recognizes a histamine site different from H_1 and H_2 and that AEBS may be, in whole or in part, a histamine or histamine-like receptor [11].

Binding studies using [^{3}H]DPPE (in place of [^{3}H]TAM) [10] and [^{3}H]histamine subsequently established that, while AEBS and histamine sites are

§ Correspondence: Lorne J. Brandes, M.D., Manitoba Institute of Cell Biology, 100 Olivia St., Winnipeg, MB R3E OV9, Canada.

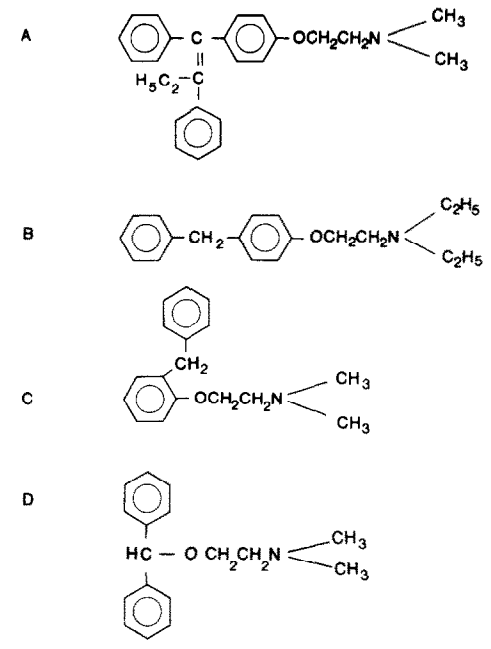

Fig. 1. Structure of (A) tamoxifen (TAM), (B) *N,N*-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine·HCl (DPPE), (C) phenyltoloxamine and (D) diphenhydramine.

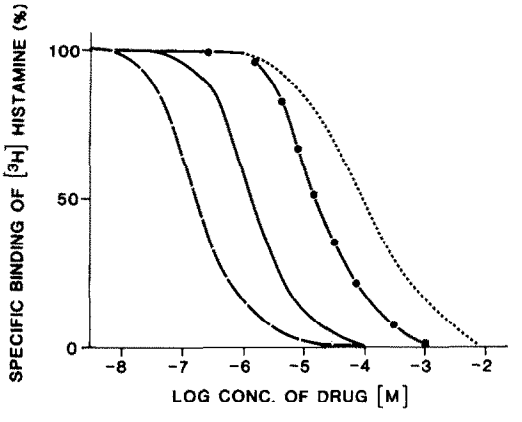

Fig. 2. Potency of histamine, DPPE, pyrilamine and cimetidine to inhibit [^{3}H]histamine binding at a single site of micromolar affinity in rat liver microsomes. Analysis of binding in rat liver microsomes, employing the LIGAND program [14], reveals a single site (K_d = $2.0 \pm 0.8 \times 10^{-6}$ M) of high capacity ($R_{max} \sim 10$ pmol/mg protein) for histamine (——). For DPPE (— —), K_i = $3.4 \pm 2.4 \times 10^{-7}$ M; for pyrilamine (●—●), K_i = $4.5 \pm 3.5 \times 10^{-5}$ M; and for cimetidine (·····), K_i = $1.0 \pm 0.8 \times 10^{-4}$ M (mean ± SE, N = 3).

not identical, there exists, both in rat brain membranes [13] and rat liver microsomes (Fig. 2), a histamine site with low (μm) affinity, different from classical H_1, H_2 and H_3 histamine receptors [15]; DPPE is to date the most potent inhibitor of histamine binding at this site. Because the rank order of potency of AEBS ligands to inhibit [^{3}H]TAM or [^{3}H]DPPE binding correlates with that to inhibit [^{3}H]histamine binding [13, 16], and because the number of DPPE/AEBS sites and micromolar histamine sites is approximately the same (pmol/mg protein), we have hypothesized that the two are closely associated; the IC_{50} effects for ligands which bind AEBS correlate with K_i values for histamine binding at a domain close by in the receptor complex [13]. Alternatively, the AEBS and histamine sites could be structurally similar but separate entities on two different proteins or protein complexes; resolution of this question will ultimately require purification of the binding moiety.

An intracellular messenger role for histamine in the mediation of platelet aggregation through binding the low affinity site identified by DPPE

Unlike other AEBS ligands, including phenothiazines and TAM [17, 18], DPPE does not bind calmodulin [19] or antagonize the action of PKC [16]. DPPE does block platelet aggregation induced by the PKC activator, PMA [16], but does *not* inhibit the phosphorylation by PKC of a protein of molecular weight 47,000 (P47) [20]. Since PMA also induces HDC activity [5], studies were performed to test the hypotheses that the non-PKC-mediated anti-PMA effects of DPPE arise from its antagonism of histamine binding, and that histamine could function as an intracellular messenger for multiple extracellular receptors [13].

Criteria that define an intracellular messenger

Five essential criteria for second messenger status have been modified from those presented by Robinson, Butcher and Sutherland [21] in their initial work with cAMP. In human platelets, each of these criteria has been satisfied to provide proof that the novel histamine site identified by DPPE is intracellular and mediates a second messenger action for newly-formed histamine [22]:

(*i*) *Physiologic agonists, at threshold concentrations for producing a physiologic response, must stimulate the formation of the substance. The formation of the proposed messenger should precede or at least coincide with, the physiologic response.*

In addition to PMA, several physiologic agonists including thrombin, platelet-activating factor (PAF) and collagen, stimulate formation of histamine in human platelets [22, 23]. With PMA, the production of histamine is coincident with the aggregatory response [22], whereas with collagen, histamine formation precedes platelet aggregation [23]. A concentration of 1.8 μg/mL collagen induces full platelet aggregation and maximum histamine stimulation (rising from a baseline level of about 12 pmol to about 40 pmol/10^9 platelets by 2 min). The lowest dose (0.4 μg/mL) of collagen that induces aggregation elicits a lesser, but still significant, increase in platelet histamine. Based on a known platelet volume of 7.3 μm^3, the mean intracellular concentration of histamine per platelet in response to PMA or collagen is approximately 3.5 to 4 μM [22], a value in keeping with the K_d value of histamine for the lower affinity site (Fig. 2).

(*ii*) *Inhibitors of the formation of the substance must block the physiologic response.*

Agonist-induced platelet aggregation is antagonized by the specific, time-dependent irreversible

inhibitor of histidine decarboxyase, α-fluoromethylhistidine (α-FMH; $IC_{50} = 10\ \mu M$), synthesized by Kollonitsch *et al.* [24], and by α-methylhistidine (α-MH; $IC_{50} = 600\ \mu M$), a reversible inhibitor. These agents block agonist-induced platelet aggregation at the same concentration at which they inhibit intracellular histamine formation [22, 23].

(*iii*) *Specific antagonists of the putative messenger should block both the binding to its receptor and the resulting physiologic response with a similar rank order of potency.*

Traditional H_1 and H_2 histamine antagonists inhibit PMA-induced platelet aggregation with significantly less potency than DPPE, the rank order correlating with that for [^{3}H]DPPE binding in rat liver microsomes and [^{3}H]histamine binding in rat brain membranes and liver microsomes [22], suggesting that newly-formed histamine, produced in response to various agonists, acts through the micromolar affinity histamine binding site identified by DPPE. An action at this novel site may explain why the antiplatelet effects of traditional antihistamines do not correlate with their potency either as H_1 or H_2 antagonists [25, 26]. However, although the order of potency for inhibition of platelet aggregation and histamine binding is similar, DPPE and the other agents are less effective as inhibitors of PMA-induced platelet aggregation [22] than as inhibitors of histamine binding (Fig. 2). This may be due, in part, to their ability to penetrate into the cell, since DPPE can inhibit collagen-induced aggregation with greater potency in permeabilized platelets ($IC_{50} = 6\ \mu M$) [23] than in non-permeabilized platelets ($IC_{50} = 30\ \mu M$). Platelets presumably sequester or metabolize DPPE since, when added 15 min before an agonist, DPPE is less effective an inhibitor than when added only 30 sec before.*

(*iv*) *The physiologic effect should be mimicked by exogenous addition of the substance at a concentration relevant to its action. For chemicals which do not readily cross membranes, the use of permeabilized cells, liposomes or permeable analogues may be required.*

Addition of histamine (0.1 to 10 μM) to permeabilized, but not intact, platelets reverses the inhibition of aggregation by HDC inhibitors and DPPE [21]. Unlike inositol triphosphate (IP_3) [27], histamine alone is not sufficient to initiate the aggregatory response in permeabilized platelets, suggesting that it must interact with one or more intermediaries; the precise nature of the complementary factor(s) is unknown at this time. The dose response for histamine to promote platelet aggregation is bell-shaped, with maximum potency at 0.1 to 1.0 μM.

(*v*) *The formation and site of action of the messenger must be shown to be intracellular.*

The reversal by histamine of inhibition of agonist-stimulated platelets by DPPE or HDC inhibitors can be demonstrated only under conditions of saponin-permeabilization [22], strongly suggesting that its site of action is intracellular. New studies [28] indicate that the histamine synthesized in human platelets in response to PMA and thrombin is not present primarily in dense granules, since conditions which release up to 90% of platelet serotonin (an amine present in these granules) are associated with retention of approximately 85% of platelet histamine. As further confirmation, approximately 90% of the histamine remains in the platelet pellet after PMA stimulation of intact platelets; however, when PMA-stimulated platelets treated with DPPE to block histamine binding are saponin-permeabilized, 75% of the platelet histamine is released into the supernatant fraction [28].

Thus, the essential criteria have been met to establish histamine as an intracellular messenger mediating platelet aggregation through binding to the novel micromolar affinity site identified by DPPE. We have suggested the terminology "H_{IC}" to designate its intracellular nature.

Mediation of multiple effects in platelets by intracellular histamine

Ultrastructural studies show that DPPE inhibits PMA-induced platelet granule membrane fusion and pseudopod formation, as well as the cell–cell attachment which occurs during aggregation, effects reversed in permeabilized platelets by histamine [29]. Such findings imply a role for intracellular histamine in the fusion of granule membranes important for secretion [30], in the cytoskeletal assembly required for pseudopod formation [31], and in the modulation of the fibrinogen receptor needed for aggregation [32]. DPPE does not inhibit phospholipase C activation nor does it antagonize the rise in cytosolic calcium in response to thrombin or the thromboxane analog EP171.† DPPE does inhibit the collagen-induced release of arachidonic acid from platelet membrane phospholipids to form thromboxane A_2, an effect reversed by micromolar quantities of histamine in permeabilized cells [23], implying that histamine may modulate phospholipase A_2. The effect of DPPE to inhibit thromboxane production does not explain all of its inhibitory effects on platelets, however. For example, even in the presence of aspirin, histamine significantly reverses the effects of DPPE to block PMA-induced aggregation,* suggesting that the effect of histamine is not solely to promote thromboxane synthesis.

Possible mediation of cellular proliferation at H_{IC} by histamine

For histamine receptor ligands, a strong correlation has been demonstrated among potency to antagonize [^{3}H]histamine binding in rat brain membranes, [^{3}H]DPPE or [^{3}H]TAM binding in rat liver microsomes, and the ability to be antiproliferative/cytotoxic to malignant cells *in vitro* [11, 13, 16]. In a 7-day assay, the IC_{50} for DPPE to inhibit the growth of MCF-7 human breast cancer cells is 5×10^{-6} M, whereas that for pyrilamine is 7.5×10^{-5} M and for cimetidine is 1×10^{-3} M [13]. Diphenylmethane compounds which lack the alkylaminoethoxy side chain, required to antagonize both [^{3}H]DPPE and [^{3}H]histamine binding, fail to affect cell proliferation at concentrations up to 1×10^{-3} M [16]. The *in vitro* antiproliferative/cytotoxic effects to MCF-7 cells of

* Saxena SP and Gerrard JM, unpublished observations.
† McNicol A and Gerrard JM, unpublished observations.

DPPE at 72 hr are reversed significantly by the addition of an excess of L-histidine or L-methionine [13], two amino acids involved in histamine metabolism. The addition of histamine itself is not effective, a finding which may be explained by the poor ability of this amine to enter most cells [33], and correlating with the demonstration that platelets must be permeabilized for histamine reversal of the anti-aggregatory effects of DPPE and HDC inhibitors [22]. However, as opposed to platelets, a role for histamine as a second messenger mediating growth is still largely inferential, and more direct evidence is required to make a definitive statement.

A possible role for intracellular histamine in gastric mucosa: Multiple effects of DPPE in the gut

Histamine is critically involved as an extracellular mediator of gastric function. Since others have suggested the existence of low affinity histamine binding sites, in addition to H_2 receptors, in, or on, gastric parietal cells [34], the effects of DPPE on gastric acid secretion and ulcerogenesis were examined, employing models of conscious gastric acid secretion under both basal and stimulated conditions, and of acute gastric ulceration, including restraint/cold stress and ethanol [35]. It was observed tht DPPE is an active agent in the gut ($ED_{50} \sim 8$ mg/kg); at a dose of 32 mg/kg, DPPE profoundly inhibits restraint/cold stress and ethanol-induced gastric ulcer formation [35], as well as cysteamine-induced duodenal ulceration [36]. Despite the fact that DPPE interacts at a site different from cimetidine and ranitidine [11, 13], it significantly reduces both basal and H_2 agonist (dimaprit)-stimulated gastric acid secretion. A lesser, but significant effect to antagonize bethanechol-stimulated gastric acid secretion has also been observed [35]. Thus, as in platelets, DPPE blocks the effects of multiple agonists in the gut.

In vitro, DPPE confers direct cytoprotection against ethanol injury in isolated parietal cells.* *In vivo*, DPPE inhibition of stress ulcer formation is abolished by a non-ulcerogenic, but prostaglandin-depleting dose of indomethacin [35], suggesting that its gastroprotection results, at least in part, from increasing endogenous prostaglandin production. Since DPPE affects prostaglandin levels in platelets, an action reversed in permeabilized cells by histamine [23], we are investigating whether its effects on prostaglandins in the gut likewise could be mediated at H_{IC} and whether, as in platelets, intracellular histamine is a second messenger mediating parietal cell function.

A second messenger role for histamine in the brain?

That histamine may function as an intracellular second messenger in the brain is suggested by binding studies showing the interaction of DPPE with low affinity histamine sites in rat brain cortex and hippocampal membranes [13]. The rank order of potency to inhibit [^{3}H]histamine binding is the same as in liver microsomes: DPPE > pyrilamine ≫ cimetidine or ranitidine. Thus, the histamine site antagonized by these ligands is neither H_1 or H_2; similarly, because of its low affinity, the site cannot be the newly-described high affinity (K_d = 1–5 nM) H_3 autoreceptor, recently described by Schwartz *et al.* [15]. Like dopamine, catecholamines, 5-hydroxytryptamine (5-HT) and acetylcholine, histamine is now accepted as a true neurotransmitter, but its exact role in brain physiology has always been difficult to assess; it has been speculated that histamine acts mainly to modulate the activity of other amines [37], a suggestion in keeping with our postulate of a second messanger action [13].

The demonstration that, like DPPE, phenothiazines and tricyclics also compete for [^{3}H]DPPE binding [19] raises the possibility that these neuroleptic agents may have major interactions at H_{IC}. Recently, we observed in mice that DPPE, in a dose-dependent fashion, diminishes vertical climbing activity.† Since spiroperidol, a dopamine (D_2) receptor antagonist with high potency [38], competes, very weakly for DPPE binding and vice versa,‡ it may be reasonable to postulate that DPPE is acting to block D_2 receptor-mediated climbing activity indirectly, through antagonizing second messenger histamine at H_{IC}. Thus, it is anticipated that studies to test the hypothesis that histamine acts as an intracellular messenger in the central nervous system, mediating the function of other primary brain receptors, may lead to fertile areas of new investigation.

Conclusion

With our discovery of H_{IC} and its role in mediating agonist-induced platelet aggregation, histamine now joins a small group of other chemicals which qualify as true intracellular messengers: cAMP, cGMP, calcium, inositol triphosphate and diacylglycerol. In addition, a strong case has been made for polyamines as intracellular messengers [39]; other amines ultimately may join this select list. Further studies are underway to define such a role for histamine in growth and in cell systems other than platelets. Ultimate acceptance of the second messenger histamine hypothesis also will depend upon independent confirmation in other laboratories, and the clarification of interactions of intracellular histamine with other second messenger and signal transduction pathways. Finally, interaction at an intracellular histamine site for traditional antihistamines, various neuroleptics and triphenylethylene-derivative antiestrogens now must be considered in the overall pharmacological profile of these agents.

Acknowledgements—This work was supported by grants from the Manitoba Health Research Council, the Winnipeg Foundation, The PHT Thorlakson Foundation, the Manitoba Cancer Treatment and Research Foundation, the Children's Hospital of Winnipeg Research Foundation, the Sellers Foundation and the Medical Research Council of Canada. F. S. L. and J. M. G. are MRC Research Scientists.

* Glavin GB, unpublished observations.

† Pinsky C, Bose R and Brandes LJ, unpublished observations.

‡ Brandes LJ and LaBella FS, unpublished observations.

REFERENCES

1. Brandes LJ and Hermonat MW, A diphenylmethane derivative specific for the antiestrogen binding site in rat liver microsomes. *Biochem Biophys Res Commun* **123**: 724–728, 1984.

2. Sutherland RL, Murphy LC, Foo MS, Green MD, Whybourne AM and Krozowski ZS, High affinity anti-estrogen binding site distinct from the estrogen receptor. *Nature* **288**: 273–275, 1980.
3. Kahlson G and Rosengren E, Histamine formation as related to growth and protein synthesis. In: *Biogenic Amines as Physiological Regulators* (Ed. Blum JJ), pp. 223–238. Prentice-Hall, Englewood Cliffs, NJ, 1970.
4. Mackay D, Reid JD and Shepherd DM, Histamine decarboxylase and growth. *Nature* **191**: 1311–1312, 1961.
5. Watanabe T, Tazuchi Y, Sasaki K, Tysuyama K and Kitamura Y, Increase in histidine decarboxylase activity in mouse skin after application of the tumor promotor tetradecanoylphorbol acetate. *Biochem Biophys Res Commun* **100**: 427–432, 1981.
6. Thastrup O, Dawson AP, Scharff O, Foder B, Cullen PJ, Drobak BK, Bjerrum PJ, Christensen SB and Havley MR, Thapsigargin, a novel molecular probe for studying intracellular calcium release and storage. *Agents Actions* **27**: 17–23, 1989.
7. Hakii H, Fujiki H, Saganuma M, Nakayasu M, Tahira T, Sugimura T, Schever PJ and Christensen SB, Thapsigargin, a histamine secretagogue, is a non-12-*O*-tetradecanoylphorbol-13-acetate (TPA) type tumor promoter in two-stage mouse skin carcinogenesis. *J Cancer Res Clin Oncol* **111**: 177–181, 1986.
8. Jensen EV, Block GE, Smith S, Kyser K and Desombre ER, Binding of estrogen in target tissues. Estrogen receptors and breast cancer response to adrenalectomy. *Natl Cancer Inst Monogr* **34**: 55–79, 1971.
9. Brandes LJ, A diphenylmethane derivative selective for the antiestrogen binding site may help define its biological role. *Biochem Biophys Res Commun* **123**: 244–249, 1984.
10. Brandes LJ and Bogdanovic RP, New evidence that the antiestrogen binding site is a novel growth-promoting histamine receptor ($?H_3$) which mediates the anti-estrogenic and antiproliferative effects of tamoxifen. *Biochem Biophys Res Commun* **134**: 601–608, 1986.
11. Brandes LJ, Macdonald LM and Bogdanovic RP, Evidence that the antiestrogen binding site is a histamine or histamine-like receptor. *Biochem Biophys Res Commun* **126**: 905–910, 1985.
12. Kroeger EA and Brandes LJ, Evidence that tamoxifen is a histamine antagonist. *Biochem Biophys Res Commun* **131**: 750–755, 1985.
13. Brandes LJ, Bogdanovic RP, Cawker MD and LaBella FS, Histamine and growth: Interaction of antiestrogen binding site ligands with a novel histamine site that may be associated with calcium channels. *Cancer Res* **47**: 4025–4031, 1987.
14. Munson RJ and Rodbard D, LIGAND: A versatile computerized approach for characterization of ligand-binding systems. *Anal Biochem* **107**: 220–239, 1980.
15. Schwartz JC, Arrang JM, Garbarg M and Korner M, Properties and roles of the three subclasses of histamine receptors in brain. *J Exp Biol* **124**: 203–224, 1986.
16. Brandes LJ, Gerrard JM, Bogdanovic RP, Lint DW, Reid RE and LaBella FS, Correlation of the antiproliferative action of diphenylmethane-derivative antiestrogen binding site ligands with antagonism of histamine binding but not of protein kinase C-mediated phosphorylation. *Cancer Res* **48**: 3954–3958, 1988.
17. Lam PHY, Tamoxifen is a calmodulin antagonist in the activation of cAMP phosphodiesterase. *Biochem Biophys Res Commun* **118**: 27–32, 1984.
18. O'Brian CA, Liskamp RM, Solomon BH and Weinstein IB, Triphenylethylenes: A new class of protein kinase C inhibitors. *J Natl Cancer Inst* **76**: 1243–1247, 1968.
19. Brandes LJ, Bogdanovic RP, Cawker MD and Bose R, Antiproliferative properties of tamoxifen and phenothiazines may be mediated by a unique histamine receptor ($?H_3$) distinct from the calmodulin-binding site. *Cancer Chemother Pharmacol* **18**: 21–23, 1986.
20. Imaoka T, Lynhham JA and Haslam RJ, Purification and characterization of the 47,000 dalton protein phosphorlyated during degranulation of human platelets. *J Biol Chem* **258**: 11404–11414, 1983.
21. Robinson GA, Butcher FR and Sutherland EW, *Cyclic AMP*, p. 36. Academic Press, New York, 1971.
22. Saxena SP, Brandes LJ, Becker AB, Simons K, LaBella FS and Gerrard JM, Histamine is an intracellular messenger mediating platelet aggregation. *Science* **243**: 1596–1599, 1989.
23. Saxena SP, McNicol A, Brandes LJ, Becker AB and Gerrard JM, A role for intracellular histamine in collagen-induced platelet aggregation. *Blood* **75**: 407–414, 1990.
24. Kollonitsch J, Patchett AA, Marburg S, Maycock AL, Perkins LM, Doldouras GA, Duggan EE and Aster SD, Selective inhibitors of biosynthesis of aminergic neurotransmitters. *Nature* **274**: 906–908, 1978.
25. Hermann RG and Frank JD, Effect of adenosine derivatives and antihistaminics on platelet aggregation. *Proc Soc Exp Biol Med* **123**: 654–660, 1966.
26. Horton MA, Amos RJ and Jones RJ, The effect of histamine H_2 receptor antagonists on platelet aggregation in man. *Scand J Haematol* **31**: 15–19, 1983.
27. Israels SJ, Robinson P, Docherty JC and Gerrard JM, Activation of permeabilized platelets by inositol-1,4,5-triphosphate. *Thrombosis Res* **40**: 499–509, 1985.
28. Saxena SP, McNicol A, Brandes LJ, Becker AB and Gerrard JM, Histamine formed in stimulated human platelets is cytoplasmic. *Biochem Biophys Res Commun* **164**: 164–168, 1989.
29. McNicol A, Saxena SP, Brandes LJ and Gerrard JM, A role for intracellular histamine in ultrastructural changes induced in platelets by phorbol esters. *Arteriosclerosis* **9**: 684–689, 1989.
30. Gerrard JM, Beattie LL, Park J-S, Israels SJ, McNicol A, Lint D and Cragoe EJ Jr, A role for protein kinase C in the membrane fusion necessary for platelet granule secretion. *Blood* **74**: 2405–2413, 1989.
31. Carroll RC, Butler RG, Morris PA and Gerrard JM, Separable assembly of platelet pseudopodal and contractile cytoskeletons. *Cell* **30**: 385–393, 1982.
32. Mustard JF, Kinlough-Rathbone RL and Packham MA, Platelet activation: An overview. *Agents Actions* (Suppl) **21**: 23–26, 1987.
33. Hosli E and Hosli L, Autoradiographic localization of binding sites for [^{3}H]histamine and H_1- and H_2-antagonists on cultured neurones and glial cells. *Neuroscience* **13**: 863–870, 1984.
34. Ekblad EBM and Licko V, Comparing binding of histamine and H_2-antagonists with their effects on gastric acid secretion. *Biochim Biophys Acta* **923**: 315–322, 1987.
35. Glavin GB and Brandes LJ, Antiulcerogenic and antisecretory effects of a novel diphenylmethane derivative and antiestrogen binding site ligand. *Can J Physiol Pharmacol* **66**: 1139–1143, 1988.
36. Glavin GB and Brandes LJ, A novel non-H_1, non-H_2 histamine antagonist protects against cysteamine-induced duodenal ulceration. *Pharmacology* **37**: 277–280, 1988.
37. Prell GD and Green JC, Histamine as a neuroregulator. *Annu Rev Neurosci* **9**: 209–254, 1986.
38. Seeman P and Niznik HB, Dopamine D_2 receptors photolabelled by [^{3}H]azido-methylspiperone. *Eur J Pharmacol* **127**: 297–299, 1986.
39. Koenig H, Goldstone AD and Lu CY, Polyamines regulate calcium fluxes in a rapid plasma membrane response. *Nature* **305**: 530–534, 1983.