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SUPPRESSION OF PASSIVE CUTANEOUS ANAPHYLAXIS BY PERTUSSIS TOXIN, AN ISLET-ACTIVATING PROTEIN, AS A RESULT OF INHIBITION OF HISTAMINE RELEASE FROM MAST CELLS

TSUTOMU NAKAMURA and MICHIO UI*†

Kyoto Research Laboratories, Kakan Pharmaceutical Co. Ltd., Yamashina, Kyoto 607, Japan, and *Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

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Abstract—Passive cutaneous anaphylaxis (PCA) produced by antigen challenge to antibody-sensitized rats were interfered with by prior treatment with pertussis toxin, an islet-activating protein (IAP). The degree of interference was dependent on the dose and injection time of IAP; the effect of IAP developed slowly, with a maximal effect being observed 3 days later. Inhibition of PCA by IAP was associated with a decrease in histamine release from peritoneal mast cells, making it very likely that the process affected was mast cell secretion. Much less histamine was discharged *in vitro*, in response to certain membrane receptor (e.g. IgE receptor) stimulation, from mast cells that had been exposed to IAP than from the cells not exposed. Such an inhibitory effect of IAP was not observed when histamine release was provoked by a calcium ionophore without mediation of membrane receptors. IAP was a stronger inhibitor of histamine release than β-adrenergic agonists. Further inhibition was produced when a β-agonist was added to IAP-treated mast cells. The increase in the cellular content of cyclic AMP was associated with β-agonist-induced, but not with IAP-induced, inhibition of histamine release. Thus, IAP inhibited histamine release by a mechanism in which metabolism of cyclic AMP was not directly involved.

The exotoxin produced by *Bordetela pertussis*, whooping cough bacteria, is known to have very diverse biological effects such as hemagglutination, lymphocytosis promotion, histamine sensitization, and adjuvant and mitogenic activities [1, 2]. In addition, the exotoxin has islet-activating activity. A protein that is responsible for this activity has been purified from the culture medium of the bacteria [3, 4]; it is referred to as islet-activating protein (IAP‡). IAP, thus prepared, is an oligomeric protein with a molecular weight of 117,000 and exhibits a sharp single band upon disc electrophoresis and by an immunodiffusion test [3]. Later studies with purified IAP have revealed that the toxin interacts directly with a variety of cell types including rat islet [5–8], rat heart [9], rat C6 glioma [10–12], rat fat [13] and mouse 3T3 [14] cells.

The purpose of this paper is to describe an effect of IAP on another cell type. Evidence is presented of marked prevention by IAP of the development of passive cutaneous anaphylaxis in rats, probably by means of inhibition of histamine release from mast cells with which IAP interacts directly.

MATERIALS AND METHODS

Materials. IAP was purified from the 2-day culture supernatant fraction of *B. pertussis* (Tohama strain, phase I), according to a procedure described elsewhere [3], and stored, until use, at 4° as a solution (0.1 mg/ml) in a vehicle consisting of 0.1 M potassium phosphate buffer (pH 7.0), 2 M urea and 1 mg/ml of bovine serum albumin. In control experiments, the vehicle was used in place of IAP. Somatostatin and A23187 were gifts from Professor N. Yanaihara, Shizuoka College of Pharmacy, and Dr. R. L. Hamill, Lilly Research Laboratories (Indianapolis, IN, U.S.A.) respectively. Reagents for radioimmunoassay of cyclic AMP were donated by the Yamasa Shoyu Co. Ltd. (Chiba, Japan). Compound 48/80, concanavalin A, bovine serum albumin and gelatin were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Anti-rat IgE rabbit antiserum was purchased from Miles Laboratories, Inc. (Elkhart, IN, U.S.A.). All other reagents were of analytical grade.

Preparation of antigen and antiserum. The ascaris antigen was prepared by 2,4-dinitrophenylation of a crude aqueous extract of *Ascaris suum* [15]. The DNP-ascaris thus prepared (1 mg), together with pertussis vaccine (10^{10} organisms), was injected into the footpads of female Wistar rats (body wt 200 ± 20 g). The rats received an additional intramuscular injection of 0.5 mg of the antigen 5 days later and were decapitated after another 3 days to obtain the anti-DNP-ascaris IgE-rich antiserum which was

† Author to whom correspondence should be addressed.

‡ Abbreviations: IAP, islet-activating protein; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; IgE, immunoglobulin E; PCA, passive cutaneous anaphylaxis; and DNP, 2,4-dinitrophenol.

stored at −80° until use. The serum, after 512-fold dilution, produced a vivid blue spot with a diameter greater than 5 mm in the 48-hr passive cutaneous anaphylaxis (see below for experimental procedures) and was used as the antiserum in the present study.

Passive cutaneous anaphylaxis (PCA). Forty-eight hour PCA was estimated with male Wistar rats (body wt 140–160 g) by the method of Ovary [16]. The antiserum prepared as above was diluted 40-fold, and 0.1 ml of the diluted antiserum was injected intracutaneously into the hair-clipped back of the rat. After 48 hr, the animal received an intravenous injection of 1 ml of saline containing 2 mg of the antigen (DNP-ascaris) and 2.5 mg of Evans Blue. The rat was decapitated 30 min later and skinned. The diameter of the blue spot where dye had leaked was read on the inner side of the skin. The Evans Blue in the spot was then extracted and quantified colorimetrically.

Determination of histamine released into the peritoneal cavity. Rats with body weights of 140–160 g were used. Release of histamine from mast cells *in vivo* was induced either by an intraperitoneal injection (in 5 ml of Hanks' solution) of compound 48/80 (5 μg) into normal rats, or by an injection of DNP-ascaris (10 mg as protein) into rats previously sensitized by the anti-DNP-ascaris antiserum (0.5 ml of the antiserum with a 1024-fold titer, intraperitoneally 2 days before). After 15 min, the rats were decapitated. The peritoneal fluid was centrifuged at 600 *g* for 10 min at 4°, and free histamine in the supernatant fraction was determined by the modified [17, 18] fluorometric method of Shore *et al.* [19].

In vitro *experiments with mast cells.* Rather big rats (body wt 270–330 g) were used for the collection of mast cells. They were anesthetized with ether and decapitated, and 15 ml of the Hepes-buffered solution containing heparin (10 units/ml) was injected into the peritoneal cavity. The Hepes solution (pH 7.4) was composed of 135 mM NaCl, 4.7 mM KCl, 1 mM $CaCl_2$, 1.2 mM KH_2PO_4, 0.6 mM $MgSO_4$, 3 mM $NaHCO_3$, 5.6 mM glucose, and 5 mM Hepes, and was supplemented with 0.1% bovine serum albumin and 0.1% gelatin. The abdomen was massaged for 1–2 min before the peritoneal cavity was opened by midline incision. Mast cells collected from the peritoneal fluid were purified to more than 95% by the albumin-gradient centrifugation method [20]. In experiments in which histamine was released by the antigen (DNP-ascaris), the crude peritoneal exudate was used, without purification, as the mast cell preparation.

Mast cells were exposed to IAP by incubating the cell suspension (5×10^6 cells) in 4 ml of the Hepes-buffered solution containing IAP under 95% O_2/5% CO_2 at 37° for 2 hr, unless otherwise specified (see Fig. 4B). The cells were then washed twice and resuspended in the fresh Hepes solution at a density of 1.5×10^5 cells/500 μl. The suspension, after preincubation for 10 min, was incubated with a histamine releaser for 10 or 15 min under the same conditions as IAP exposure. The incubation was terminated by the addition of 1 ml of ice-cold Tris buffer (25 mM Tris, 123 mM NaCl, 2.7 mM KCl, pH 7.4), and the supernatant fraction (0.5 ml) obtained by centrifugation for 2 min at 1400 *g* was stored at −20° before analysis for histamine. A batch of the mast cell suspension frozen before incubation was used for determination of cellular histamine content; it was subjected to three cycles of freezing and thawing and then to boiling for 5 min before the histamine analysis.

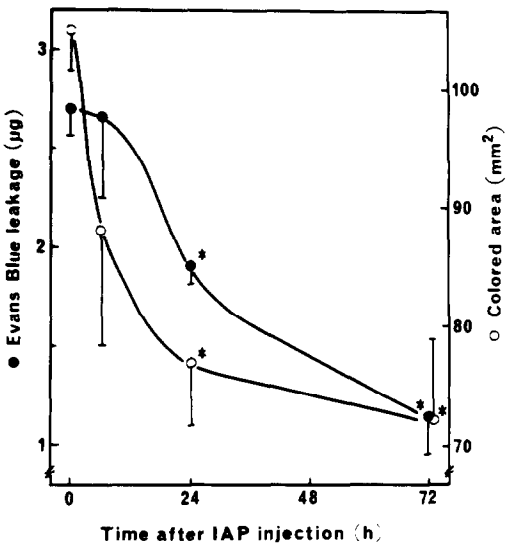

Fig. 1. Time-dependent inhibition of 48-hr PCA after treatment of rats with IAP. Rats were injected intravenously with 2 μg IAP and, after the time indicated on abscissa, received the antigen challenge in the 48-hr PCA test as described under Materials and Methods. A series of experiments with three animals for each point was repeated twice, and the mean ± S.E.M. from the six observations was obtained for the area of the blue color spot on the skin and for its dye content. Key: (*) difference from the 0-time value was significant ($P < 0.05$).

In some experiments, mast cell suspension was analyzed for the cyclic AMP content. In these cases, incubation was terminated by the addition of 0.2 N HCl followed by heating at 100° for 10 min [5], and the supernatant fraction obtained by centrifugation at 700 *g* for 5 min was subjected to radioimmunoassay by the method of Honma *et al.* [21].

RESULTS

Inhibition by IAP of 48-hr *PCA in rats.* Antigen–antibody interaction provokes an increase in capillary permeability, which is visualized by the appearance of a blue spot on the skin in response to the antigen challenge to the antibody-sensitized rats in the usual 48-hr PCA test. IAP, injected at various time intervals prior to the antigen injection, reduced the increase in permeability. It was effective when injected 24 hr before the challenge, and the effect became more pronounced 3 days later (Fig. 1). The inhibition of PCA by IAP was dose-dependent; the minimum effective dose appeared to be 0.1 to 0.3 μg/animal, when it was injected 3 days before the antigen injection (Fig. 2).

Inhibition by IAP of histamine release in vivo. Since one of the factors responsible for PCA is histamine that is discharged from antigen-stimulated mast cells, measurement was next made of histamine

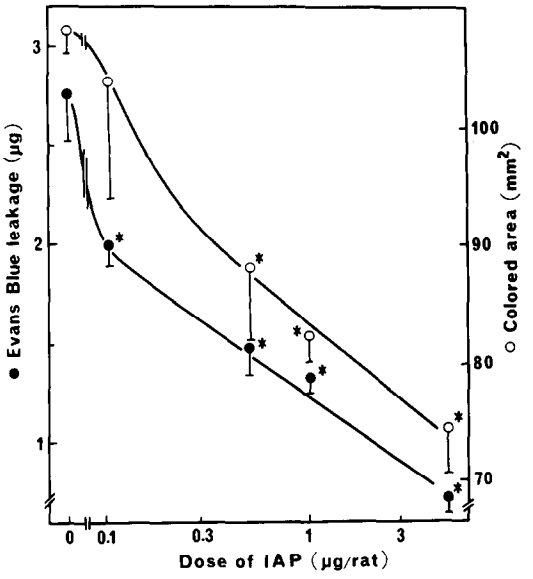

Fig. 2. Dose-dependent inhibition of 48-hr PCA by IAP. Rats were injected with the doses of IAP shown on the abscissa 3 days before the antigen challenge in the 48-hr PCA test. Experimental protocol and expression of results are the same as in Fig. 1. Key: (*) difference from the control was significant ($P < 0.05$).

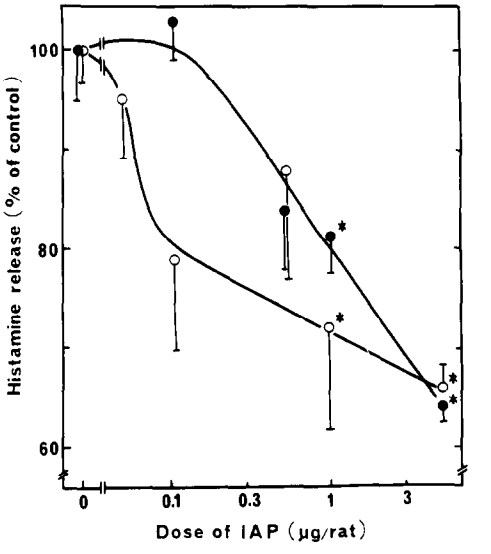

Fig. 3. Effect of IAP on histamine release *in vivo* into the peritoneal cavity in response to compound 48/80 or antigen. Normal or antiserum-sensitized rats were challenged with compound 48/80 (○) or the antigen (●), respectively, as described under Materials and Methods. IAP had been injected intravenously into these rats 3 days before the challenge. The histamine released in the rats not treated with IAP was 13.2 ± 0.43 μg/rat in response to compound 48/80, 6.0 ± 0.33 in response to the antigen, and 0.14 ± 0.01 spontaneously. Histamine released in IAP-treated rats is expressed as a percentage of these control values. A series of experiments with three animals for each point was repeated twice, and the mean ± S.E.M. from the six observations is plotted against the dose of IAP. Key: (*) difference from the control was significant ($P < 0.05$).

released into the peritoneal cavity in response to the antigen challenge to sensitized rats (Fig. 3). Prior treatment of rats with IAP decreased histamine release in PCA in a dose-dependent manner. The injection of compound 48/80, a potent histamine releaser, into normal rats caused more accumulation of histamine in the peritoneal fluid than did the antigen injection (see the legend to Fig. 3). This histamine release was also prevented by IAP; the effect of IAP was statistically significant at doses higher than 1 μg, although the dose–response relationship was not as obvious as in the case of the PCA reaction.

To determine whether the IAP-induced decrease in the histamine content of the peritoneal fluid reflected, in fact, its decreased liberation from mast cells, we collected mast cells from IAP-treated rats and estimated the release of histamine therefrom *in vitro* in response to compound 48/80 (Table I). The total content of histamine in mast cells was not affected by IAP treatment of rats, nor was its spontaneous release altered. The addition of compound 48/80 to the incubation medium caused a marked discharge of histamine from mast cells during incubation for 10 min. The amount of histamine discharged under these conditions was much less with mast cells isolated from IAP-treated rats than with the cells from non-treated control rats. Thus, the injection of IAP into rats resulted in suppression of histamine release from mast cells upon stimulation.

Inhibition by IAP in vitro *of histamine release from*

Table 1. Effect of IAP *in vivo* on compound 48/80-induced histamine release from mast cells *in vitro**

Treatment of rats	Histamine (μg/1.5 × 10^5 cells)		
	Total content	Released with	
		None	Compound 48/80 (% of total)
Vehicle	3.9 ± 0.40	0.05 ± 0.01	2.6 ± 0.23 (66 ± 1)
IAP	4.0 ± 0.35	0.04 ± 0.01	1.5 ± 0.13† (39 ± 2)

* Mast cells were collected from rats intravenously injected with vehicle or 5 μg IAP 3 days before. The cells were incubated with or without 0.5 μg/ml of compound 48/80 for 10 min. Data are means ± S.E.M. from three observations.

† The effect of IAP treatment was significant ($P < 0.01$).

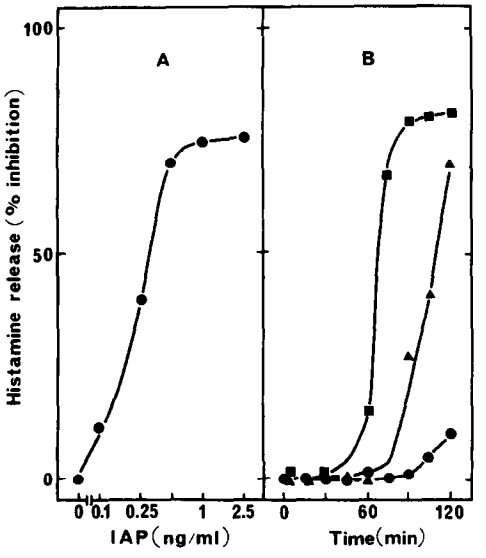

Fig. 4. Inhibition by IAP of histamine release from mast cells *in vitro* in response to compound 48/80. Mast cells were exposed to various concentrations (shown on abscissa) of IAP for 2 hr (A), or to 0.1 (●), 1 (▲) or 10 (■) ng/ml of IAP for various lengths (shown on abscissa) of time (B). These cells were then incubated with 0.5 μg/ml of compound 48/80 for 10 min to measure histamine release as described under Materials and Methods. The percent inhibition caused by IAP was calculated, and the mean value from duplicate observations is shown in each panel.

mast cells. As shown above, IAP injected into rats exerted *in vivo* a sustained influence on mast cells. It was shown that the histamine secretory response *in vitro* of the cells to stimulants, even after washing of cells, was maintained in a suppressed state after IAP treatment *in vivo*. A direct interaction of IAP with mast cells was verified by the experiments *in vitro* shown in Fig. 4. Histamine release from mast cells in response to compound 48/80 was suppressed by prior exposure *in vitro* of the cells to IAP for 2 hr. The suppression was strictly dependent on the concentration of IAP added; the concentration required for half-maximal suppression was as low as 0.25 ng/ml. The maximum inhibition caused by 1 ng/ml reached 70–80% in this experiment. (A stronger and almost total inhibition was observed in other experiments as exemplified in the inset of Fig. 5.)

Prior exposure of mast cells to IAP was required for the toxin to display its inhibitory action on histamine release. When IAP was added simultaneously with compound 48/80 to the incubation medium, the amount of histamine released was as much as that released in the absence of IAP. When cells were in contact with 10 ng/ml of IAP, its action to inhibit histamine release occurred after an apparent lag period of 30 min and the inhibition developed progressively as the time of preincubation with IAP was prolonged to 90 min (Fig. 4B). The lower the concentration of IAP used, the more delayed was the

Table 2. Effect of IAP *in vitro* on histamine release from mast cells in response to various secretagogues*

Secretagogues	IAP (ng/ml)	Histamine release by secretagogues: % of Total content	Histamine release by secretagogues: % Inhibition by IAP
Compound 48/80	0	42.4 ± 1.5	
(0.5 μg/ml)	0.5	17.5 ± 0.7†	59 ± 1.7
	1	11.4 ± 0.6†	73 ± 1.5
Antigen (1 mg/ml)	0	25.9 ± 4.8	
	1	21.6 ± 1.6	17 ± 6.2
	10	19.6 ± 0.8†	24 ± 2.9
Anti-IgE	0	32.6 ± 2.8	
(10-fold diluted)	1	21.8 ± 1.1†	33 ± 3.3
	10	20.5 ± 1.2†	37 ± 3.8
Concanavalin A	0	51.3 ± 2.2	
(10 μg/ml)	1	44.2 ± 2.2†	14 ± 4.9
	10	33.6 ± 2.0†	35 ± 3.8
Somatostatin	0	57.9 ± 2.4	
(10 μg/ml)	1	3.5 ± 1.5†	94 ± 2.6
ATP (20 μM)	0	58.0 ± 4.5	
	10	58.1 ± 4.3	
A23187	0	72.5 ± 4.8	
(0.5 μg/ml)	1	74.1 ± 2.0	
	10	70.3 ± 1.2	3 ± 1.7

* Mast cells exposed to IAP for 2 hr were incubated with secretagogues for 10 min. When the antigen, anti-Ige antibody or concanavalin A was used as a secretagogue, the cells had been sensitized by a 1-hr preincubation in the 10-fold diluted anti-DNP-ascaris antiserum (with a 1024-fold titer) and then incubated with the secretagogue in the presence of 50 μg/ml of phosphatidylserine. In this case, the incubation time was prolonged to 15 min. Data are means ± S.E.M. from three observations.

† The effect of IAP was statistically significant ($P < 0.01$).

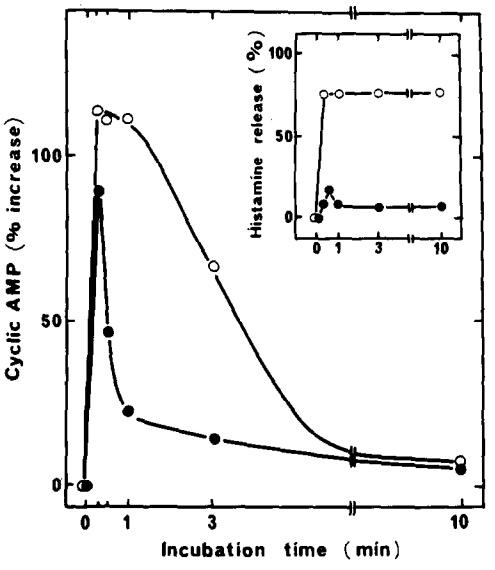

Fig. 5. Histamine release and cyclic AMP accumulation in mast cells in response to compound 48/80 and the inhibition by IAP treatment of the cells. Mast cells that had been treated with (●) or without (○) IAP (10 ng/ml) for 2 hr were incubated with 0.5 μg/ml of compound 48/80 for the time indicated on the abscissa to estimate histamine release (inset) and cyclic AMP content. The cellular contents (per 10^6 cells) of histamine and cyclic AMP before incubation were 23.2 + 1.0 μg and 284 ± 24 fmoles for control and 23.3 ± 1.7 μg and 329.39 fmoles for IAP-treated cells respectively. The amount of histamine released and the increase in cyclic AMP are expressed as percentages of these preincubation values. Each point is the mean of duplicate observations.

onset of the IAP action. For instance, the degree of inhibition caused by 0.1 ng/ml of IAP in a 2-hr preincubation was roughly comparable to the inhibition elicited by 10 ng/ml after on 1 hr (Fig. 4B).

IAP inhibited histamine secretion even if agents other than compound 48/80 were used as histamine secretagogues (Table 2). The stimulation of IgE receptors, located on the surface of sensitized mast cells, by means of its specific antigen or the anti-IgE antibody provoked histamine release, which was inhibited significantly by prior treatment of the cells with IAP. Likewise, IAP treatment of cells was effective in inhibiting their histamine secretory responses to concanavalin A or somatostatin. The IAP-induced inhibition was extraordinarily pronounced when the secretion was stimulated by somatostatin. In sharp contrast, ATP or A23187, a calcium ionophore, caused histamine secretion which was not prevented by IAP.

Table 3. Effect of β-adrenergic agonists on histamine release and cyclic AMP in control and IAP-treated mast cells*

	Histamine release (% of total)		Cyclic AMP (fmoles/10^6 cells)	
Addition	Control	IAP	Control	IAP
None	42.0	12.6	250	279
Epinephrine	20.2	4.7	470	501
Norepinephrine	25.8	5.5	416	429
Salbutamol	20.2	4.0	483	509

* Mast cells exposed to IAP or vehicle (control) for 2 hr were incubated with 10 μM epinephrine, norepinephrine or salbutamol for 30 sec. An aliquot was withdrawn for analysis of cyclic AMP. The residual aliquot was further incubated with compound 48/80 (0.5 μg/ml) for another 10 min to be analyzed for histamine release. Histamine release caused by compound 48/80 is shown in the table as a percentage of the total content, together with the cyclic AMP data immediately before the addition of the compound. Data are means from duplicate observations.

Changes in the cellular content of cyclic AMP in response to compound 48/80. When compound 48/80 was added to the mast cell suspension, the cellular content of cyclic AMP increased very sharply (Fig. 5). It reached the highest level at as early as 20 sec, the shortest time of incubation performed, and then declined gradually, returning to almost the prestimulated level 10 min later. In accordance with this change in cyclic AMP, histamine release reached the maximum level at 20–30 sec after the start of incubation (Fig. 5, inset).

The same experiments were repeated with mast cells that had been exposed to IAP for 2 hr. Cyclic AMP increased in response to compound 48/80 in IAP-treated cells as well, but the peak level was lower and the return to the basal level was much more rapid as compared to the changes observed in non-treated control cells. This time course of cyclic AMP changes is in agreement with that of histamine release. Compound 48/80 produced only a small and transient increase in histamine release in IAP-treated cells (Fig. 5, inset).

Additive effects of IAP, and β-adrenergic agonists on histamine release. Epinephrine, norepinephrine and salbutamol inhibited compound 48/80-induced histamine release (Table 3). The inhibition was associated with increases of the cellular content of cyclic AMP. Both actions of these catecholamines were due to stimulation of β-adrenergic receptors, since they were efficiently antagonized by the simultaneous addition of propranolol (data not shown). It is very likely that the increase in cyclic AMP in mast cells is responsible for β-receptor-mediated inhibition of histamine release.

Treatment of mast cells with IAP was more effective than β-adrenergic agonists in inhibiting histamine release (Table 3). The concentrations of IAP and agonists used in Table 3 were those that caused the maximum inhibition. [The concentration of these agonists required for half-maximal inhibition was less than 1 μM (data not shown), and that of IAP as shown in Fig. 4.] The combined addition of β-adrenergic agonists with IAP caused an "additive" effect; the agonists were similarly effective on IAP-treated cells as well as on non-treated cells. The results imply that IAP and β-adrenergic agonists inhibited compound 48/80-induced histamine release by different mechanisms. Actually, IAP did not alter the degree of the agonist-induced increases in cellular cyclic AMP immediately before the addition of compound 48/80. Probably, IAP inhibits histamine release by a mechanism in which changes in the cellular cyclic AMP level are not directly involved.

DISCUSSION

The present results show that pertussis toxin interacted directly with mast cells in such a fashion as to prevent the cells from releasing histamine in response to a variety of stimuli. The interaction occurred *in vitro* as well as *in vivo*. The effect of this IAP on mast cells bears a striking resemblance to its action on other cell types such as islet [7], heart [9], C6 glioma [10] and 3T3 [14] cells in that a definite lag time invariably preceded the onset of the action on cells. Recent analysis of the subunit structure of IAP revealed that IAP, an oligomeric protein, is one of the A-B toxins [22] in which the B (Binding)-oligomer binds to a particular receptor site on the cell membrane to permit the A(Active)-protomer to reach, by entering the cell through the membrane, to the intracellular site of its action [23]. Thus, the biological action of an A-B toxin is characterized by a lag period which reflects the time required by the A-component to transverse the plasma membrane. It is very likely, therefore, that the A-protomer of IAP is the real factor which, by entering mast cells, is responsible for the inhibition of histamine discharge. In any case, the effect *in vivo* of IAP to suppress the PCA reaction may have arisen from the inhibitory action of its A-protomer on sensitized mast cells.

One of the additional unique properties of IAP was the long duration of its action. The greatest effect *in vivo* was observed 3 days after IAP injection (Fig. 1). The effect became weaker gradually afterwards, but was still evident 10–15 days later (not shown), as previously reported for its action *in vivo* on insulin secretory responses of rats [4]. In experiments *in vitro* as well, the action of IAP on cells lasted for a long time as observed with cultured 3T3 cells [14]. It has been reported recently that the A-protomer of IAP, like the A-components of diphtheria [24] and cholera [25] toxins, catalyzed ADP-ribosylation of a membrane protein [23]. This ADP-ribosylation of a protein appears to be closely related to the mechanism whereby IAP acts on mast cells as well as on other cell types [11–14, 23]. Probably, the action of IAP was durable because of the high stability of an ADP-ribosyl bond once formed [24]. Likewise, the fact that the higher the concentration of IAP used, the more rapidly the action of IAP occurred (Fig. 4B) may be explained on the basis of its action as a catalyst of ADP-ribosylation.

IAP-induced inhibition of histamine secretion *in vitro* was observed only when the secretion occurred upon stimulation of Fc-receptors by the specific antigen or the anti-IgE antibody or other membrane receptors by compound 48/80 or sematostatin. IAP was without effect on the secretion caused by ATP or A-23187, probably bypassing particular receptor stimulation, as a rather direct result of Ca^{2+} entrance into the cell [26, 27]. Nor was it effective on spontaneous release of the amine. Such characteristics are very similar to those observed in its actions on other cell types so far reported; interaction of IAP with islet [5], cardiac [9] or C6 glioma [10] cells caused enhancement of receptor-mediated increases, or attenuation of receptor-mediated decreases, in the cellular cyclic AMP content, without alteration of the baseline content maintained in the absence of receptor stimulation.

The addition of compound 48/80 to mast cells gave rise to a rapid increase in cellular cyclic AMP (Fig. 5). This increase in cyclic AMP does not appear to trigger histamine discharge, since cyclic AMP is known to inhibit, rather than stimulate, histamine release from mast cells [28–30]. Conceivably, compound 48/80-induced histamine release was associated with an increase in cyclic AMP as a secondary or compensatory reaction, although no adequate explanation is currently available as to how the compound increased cyclic AMP in mast cells. In any case, IAP-induced inhibition of histamine release did not result from increases in cyclic AMP. Conversely, less cyclic AMP increased in IAP-treated mast cells than in control non-treated cells in response to compound 48/80, presumably because of much less histamine release from the treated cells.

Failure of IAP to increase cyclic AMP was demonstrated further in Table 3. In contrast to β-adrenergic agonists that inhibited compound 48/80-induced histamine release as a result of increases in cyclic AMP, IAP was without effect on the cyclic AMP content of mast cells. Nor did it affect the β-agonist-induced increases in the nucleotide content. Thus, IAP does not share the same mechanism as β-adrenergic agonists in inhibiting histamine release. In fact, the inhibition of histamine release caused by IAP was "additive" to the inhibition elicited by β-agonists when both agents were added together to the mast cell suspension. Thus, IAP appears to inhibit compound 48/80-induced histamine release from mast cells by a mechanism in which cyclic AMP is not directly involved.

IAP inhibited histamine release in different magnitudes when different secretagogues were used (Table 2). The inhibition was much greater with compound 48/80 than with stimulants of Fc receptors or concanavalin A. The mechanism for IAP-induced inhibition might differ in these cases. The strongest inhibition was provoked by IAP when somatostatin was used as a histamine releaser. Somatostatin stimulates mast cell secretion via the decrease in cyclic AMP in the cell [31]. The inhibition of somatostatin-induced histamine release by IAP is consistent with the previous findings that somatostatin-induced decreases in cyclic AMP in rat islet cells were interfered with by prior treatment of islet-donor rats with IAP [5]. Many more studies are required before any conclusion can be reached as to how IAP interacts with mast cells, affecting their histamine secretory responses to a variety of secretagogues.

The inhibition by IAP *in vivo* of a PCA reaction (Figs. 1 and 2), as well as the accompanying histamine release (Fig. 3), was statistically significant but of rather modest magnitude. It is in accordance with the small inhibition *in vitro* of histamine release that was induced by stimulants of IgE receptors (Table 2). This modest inhibitory action of IAP may be related to the findings that pertussis toxin makes rodents more, rather than less, sensitive to anaphylaxis. Pertussis toxin or IAP enhances histamine-induced death of mice or rats [4]. Yajima *et al.* [32] reported that histamine acts as a strong insulin

secretagogue and causes severe hypoglycemia in IAP-treated rodents as a result of IAP-specific enhancement of their insulin secretory response to the amine. Hypoglycemia was responsible for exaggerating the toxic action of histamine or decreasing the lethal dose of it. In anaphylaxis, therefore, less histamine should be released but its toxic action must be much stronger in IAP-treated than in normal rodents.

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