Islet-activating Protein
A MODIFIER OF RECEPTOR-MEDIATED REGULATION OF RAT ISLET ADENYLATE CYCLASE*

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Adenylate cyclase of the membrane-rich fraction of 24-h cultured islets was inhibited by epinephrine via α-adrenergic receptors. Epinephrine was inhibitory only when the enzyme was activated by GTP; the degree of inhibition was highly proportional to the degree of GTP activation. Adenylate cyclase of islets cultured with islet-activating protein (IAP), one of the pertussis toxins, was less susceptible to epinephrine inhibition. The degree of inhibition was markedly reduced without changes in potency of the catecholamine and in GTP dependence after IAP treatment. None of the other kinetic properties of the enzyme including the affinity for substrate, sensitivity to guanine nucleotide and fluoride activation, and cholora toxin-induced modification of enzymic activity were affected by treatment of islets with IAP, suggesting that neither the catalytic nor the GTP-regulatory component of the membrane adenylate cyclase complex is the site of IAP action. Slight activation of the enzyme by glucagon or adenosine tended to be enhanced by IAP treatment. Thus, a mechanism whereby membrane receptors are linked to adenylate cyclase appears to be modified by exposure of islet cells to IAP.

Islet-activating protein is a new protein isolated from the culture medium of Bordetella pertussis (1, 2) as one of the pertussis toxins (3, 4). It interacts with islet cells slowly (5) to give rise to striking reversal of α-adrenergic inhibition of cAMP accumulation in, and consequent insulin release from, the islet cells (5, 6). Moreover, the accumulation of cAMP associated with insulin release enhanced by various insulin secretagogues was greater in the cells treated with IAP than in control cells not treated, when breakdown of the cellular cAMP was prevented by a methylxanthine (5-7). There was no difference, however, in the cAMP content between IAP-treated and control cells unless its generation was stimulated or inhibited by the addition of receptor agonists. Possibly, IAP would exert its unique influences on the mechanism by which stimulation of membrane receptors leads to inhibition or activation of adenylate cyclase in islet cells.

The purpose of the present paper is to substantiate this possibility by studying receptor-mediated regulation of adenylate cyclase activity in the membrane preparation from islets that had been cultured with IAP. We show that GTP-dependent inhibition of adenylate cyclase by epinephrine was markedly attenuated, and its activation by other types of agonists was somewhat potentiated by the pretreatment of islet cells with IAP.

EXPERIMENTAL PROCEDURES

Materials---IAP was purified by our colleagues (R&D Project, Kagoshima, Japan) from the 3-day culture supernatant of B. pertussis (Tohama strain, phase 1) according to the procedure described elsewhere (1). ATP is the Sigma product (catalog No. A2383) prepared by phosphorylation of adenosine. GTP, GDP, adenosine, creatine phosphate (Na or Tris salt), and creatine phosphokinase were also obtained from Sigma; App(NH)p and Gpp(NH)p were from Boehringer Mannheim; cholera toxin was from Chemo-Serv Therapeutic Research Institute (Kumamoto, Japan). Ro-20-1724 was supplied through the courtesy of Dr. H. Shimizu, Nippon Roche Research Center (Kamakura, Japan). The sources of all other materials used throughout are those described in the previous papers (5, 6).

Crude Membrane Preparation from Cultured Islets---Rat islets were isolated by the collagenase method (6, 8) and cultured with or without IAP (100 ng/ml) for 24 h as described elsewhere (5). Usually 200 islets (obtained from 2-3 male Wistar rats) were put in a sterile plastic Petri dish (60 mm in diameter) which contained 4 ml of the TCM 199 culture medium (Earle’s salt) supplemented with 10% heat-inactivated fetal calf serum, antibiotics, 16.7 µM glucose, and 200 kallikrein inhibitory units/ml of aprotinin. The dish was stored in a humidified air containing 5% CO₂ at 37 °C. The protein content of this preparation was about 0.25 mg/ml. All procedures were performed at 0-4 °C.

Adenylate Cyclase Assay---The adenylate cyclase activity was measured by following the synthesis of cAMP from nonradioactive ATP, from [3H]ATP, or from [3H]App(NH)p.

at concentrations indicated in figures. The concentration of Na was maintained between 25 and 50 mM except when otherwise indicated in Fig. 7. Incubation was initiated by the addition of the membrane preparation (1–2 μg of protein) and continued for 10 min (unless otherwise specified) at 37 °C in a total volume of 25 μl. The cAMP synthesis was terminated by the addition of 10 μl of 0.33 M ZnSO₄ and 10 μl of 0.5 M Na₂CO₃. The precipitate formed after thorough mixing with a Vortex mixer followed by standing for 1 h was centrifuged off at 1500 × g for 10 min. A sensitive and specific radioimmunoassay method for cAMP was then applied to 30 μl of the supernatant.

This procedure for reaction termination was adopted as a simple method for separation of generated cAMP from ATP and other nucleotides according to Chan and Lin (11). A recovery test detected 0.9 ± 0.08% of [³H]ATP (added at 1 mM) and 95 ± 0.6% of [³H]cAMP (10 mM) added to the reaction mixture in the supernatant fraction (number of observations, 5). Since the cross-reactivity of anti-cAMP antiserum used in the radioimmunoassay was negligible (less than one-milligram percent) with ATP or GTP (10), cAMP generated from an excess amount of ATP (up to 1 mM) in the presence of GTP was safely estimated by the present method.

The experimental results are reported as picomoles of cAMP synthesized/min/mg of protein. All experiments were performed in duplicate or in triplicate and were repeated two or three times with different batches of the cell-free preparations.

RESULTS

Inhibition of Adenylyl Cyclase by Epinephrine and its Attenuation by Treatment of Islets with IAP—Cyclic AMP was generated progressively when the membrane preparation from cultured islets was incubated with ATP or App(NH)p; the generation proceeded almost linearly up to 20 min in the presence or absence of enzyme activators such as guanine nucleotides or fluoride ion.² These activators have been reported to be effective on adenylyl cyclases from rat (12–16), mouse (17), and hamster (18) islet cells. The enzyme was located almost exclusively in the cell membrane because much less cAMP was generated when the particulate-free supernatant was used instead of the membrane-rich pellet (data not shown).

Fig. 1 shows adenylyl cyclase activity measured in the presence of GTP as a function of concentrations of ATP or App(NH)p. The maximal enzyme velocity obtained with saturating concentrations of substrate was much smaller with App(NH)p than with ATP, but the Km value was in the same order of magnitude in either case. Plotting these data according to Lineweaver-Burk⁶ afforded the Km value of around 50 μM which is comparable to the value previously reported by Johnson et al. (14). There was no significant difference in the Km and Vmax values for App(NH)p as well as for ATP between enzymes of islets cultured with and without IAP.

Epinephrine added to the enzyme assay mixture inhibited islet adenylyl cyclase. The inhibition was of a noncompetitive type in the sense that the percentage of inhibition was maintained essentially unaltered regardless of the concentration of substrate, as illustrated by dotted lines in Fig. 1, A and C. Pretreatment of islets with IAP rendered the adenylyl cyclase less susceptible to epinephrine inhibition; the degree of inhibition was much smaller for the enzyme of IAP-treated islets than for the enzyme of nontreated islets at any concentration of substrate used (Fig. 1, B and D). Thus, the Vmax value of adenylyl cyclase was reduced by epinephrine addition and recovered by IAP pretreatment of cells without significant changes in its Km value by these treatments.

Progressive inhibition of adenylyl cyclase by increasing concentrations of epinephrine is illustrated in Fig. 2 in which the membrane preparations obtained from four batches of islets cultured without IAP and from four additional batches cultured with IAP were assayed for adenylyl cyclase separately, and the results are plotted as means ± S.E. from these respective four preparations. The concentration of epinephrine required to cause the half-maximal inhibition was around 0.1 μM. It was only slightly higher than the catecholamine concentration which elicited the half-maximal diminution of the cellular content of cAMP when added to the incubation medium of intact islet cells (6). The inhibition was significantly reduced by the IAP treatment at any concentration of epinephrine without significant changes in its concentration.
which causes the half-maximal inhibition.

Epinephrine-induced inhibition of adenylate cyclase was mediated via \( \alpha \)-adrenergic receptors, as revealed by attenuation of the inhibition upon the simultaneous addition of phenolamine, an \( \alpha \)-adrenergic antagonist. The extent of the \( \alpha \)-adrenergic antagonist, in sharp contrast with the action of IAP, was competitive with epinephrine; the progressive curve as shown in Fig. 2 was shifted to the right in the presence of 10 \( \mu \)M phenolamine to result in a 500-fold increase in the catecholamine concentration to cause the half-maximal inhibition of the enzyme (data not shown).

**Essential Role of GTP in IAP-susceptible Epinephrine Inhibition of Adenylate Cyclase**—Adenylate cyclase activity as well as its inhibition by epinephrine was highly dependent on the presence of guanine nucleotides. The effect of Gpp(NH)p is shown in Fig. 3 and that of GDP in Fig. 4, both in comparison with the effect of GTP. App(NH)p was replaced for ATP, and no ATP-regenerating system was added in the latter case for GDP to be prevented from being phosphorylated to GTP. Adenylate cyclase activity increased progressively as the concentrations of these guanine nucleotides were increased; Gpp(NH)p was as effective as, and GDP was less effective than, GTP in this regard. There was no difference in adenylate cyclase activity between IAP-treated and control islets regardless of whether guanine nucleotides were added or not. A striking difference was observed between the effects of guanine nucleotides when adenylate cyclase was assayed in the presence of epinephrine. Epinephrine was inhibitory only when the reaction mixture was supplemented with GTP. No inhibition was evoked if Gpp(NH)p or GDP was replaced for GTP. The epinephrine inhibition was dependent on GTP concentrations; the higher the concentration that was used, the greater was the degree of percentage of inhibition (Figs. 3 and 4, lower panels). The addition of NaF at 10 mM caused activation of adenylate cyclase to an extent comparable to that caused by 1 \( \mu \)M GTP. Neither IAP treatment of islets nor epinephrine addition to its membrane preparation modified adenylate cyclase in the presence of NaF.

The action of IAP treatment was observed only when epinephrine exerted its inhibitory action in the presence of GTP; the degree of epinephrine inhibition was much smaller in the membrane from IAP-treated islets than from non-treated islets. Dependence of epinephrine inhibition on GTP concentrations was slightly observed even in IAP-treated islets when ATP was used as substrate (Fig. 3D), but it was not evident in the presence of App(NH)p because of almost complete abolition of epinephrine inhibition by IAP.

The submaximal doses (0.1 and 1 \( \mu \)M) of Gpp(NH)p not only caused a partial activation of adenylate cyclase by themselves but also interfered with the enzyme activation evoked by GTP simultaneously added (Fig. 5, A and B). The interference was so severe that GTP was no longer effective in the presence of 1 \( \mu \)M Gpp(NH)p. Virtually the same results were obtained whether islets had been cultured with or without IAP. The noncompetitive inhibition patterns in Fig. 5 may reflect the fact that Gpp(NH)p is tightly bound to the GTP site on the regulatory subunit of membrane adenylate cyclase. In contrast, GTP could be readily detached from the binding site after being hydrolyzed to GDP as a “turn-off” reaction of the enzyme activation (19, 20). It is unlikely that Gpp(NH)p is bound to different sites because Gpp(NH)p-induced activation of adenylate cyclase was inhibited by GDP in a competitive manner in the membrane preparations from islets cultured with or without IAP (Fig. 6). Epinephrine-induced inhibition of adenylate cyclase became smaller in magnitude as the action of GTP was suppressed by Gpp(NH)p (Fig. 5C).
Control of Adenylate Cyclase by Islet-activating Protein

Inhibition of Adenylate Cyclase—When the concentration of Na⁺ was kept at 3 mM or lower in the reaction mixture, epinephrine failed to inhibit adenylate cyclase even in the presence of GTP (Fig. 7A). Increases in the Na⁺ concentration beyond 10 mM caused slight activation of adenylate cyclase and concomitantly enabled the catecholamine to inhibit the enzyme progressively. The activation of the enzyme and its inhibition by epinephrine were almost maximal when the Na⁺

Under these conditions, too, epinephrine was less effective in IAP-treated islets than in control islets. The activation of adenylate cyclase by GTP appears to be essential for the enzyme to be susceptible to epinephrine inhibition which is readily overcome by pretreatment of islet cells with IAP.

Sodium Ion Requirement for IAP-susceptible Epinephrine

FIG. 5. Adenylate cyclase activity as a function of GTP in the presence or absence of Gpp(NH)p. Membrane fractions prepared from islets cultured with (B and D) or without (A and C) 100 ng/ml of IAP were assayed for adenylate cyclase with 0.5 mM ATP, an ATP-regenerating system, and increasing concentrations of GTP (m refers to absence of GTP). Assay was performed in the presence (A, 0.1 pM; B, 1 pM) or absence (C, D) of Gpp(NH)p. The percentage of inhibition caused by 10 μM epinephrine is shown in C and D.

FIG. 6. Competitive inhibition of Gpp(NH)p-activated adenylate cyclase by GDP. Membrane fractions prepared from islets cultured with (B) or without (A) 100 ng/ml of IAP were assayed for adenylate cyclase with 1 mM App(NH)p and increasing concentrations of Gpp(NH)p (m refers to absence of Gpp(NH)p). No ATP-regenerating system was added. The incubation medium was supplemented with 1 μM GDP (0) or not (C).

FIG. 7. Effect of NaCl on epinephrine-induced inhibition of adenylate cyclase. Membrane fractions prepared from islets cultured with (B) or without (A) 100 ng/ml of IAP were assayed for adenylate cyclase with 0.25 mM ATP, 10 μM GTP, and an ATP-regenerating system (Tris salt of phosphocreatine was used instead of its Na salt) in the presence of increasing concentrations of NaCl. Assay was performed with (0) or without (C) 10 μM epinephrine.

FIG. 8. Effects of GTP and Gpp(NH)p on adenylate cyclase and its inhibition by epinephrine in membrane fractions of cholera toxin-treated islets. Islets were cultured with (B and D) or without (A and C) 100 ng/ml of IAP for 24 h. Cholera toxin (1 μg/ml) was added to all culture dishes 2 h before the end of culture. Membrane fractions prepared from these cultured islets were assayed for adenylate cyclase with 0.5 mM ATP and an ATP-regenerating system in increasing concentrations of GTP (0, C) or Gpp(NH)p (A, D). m refers to the absence of guanine nucleotide. Assay was performed with (0, A) or without (C, D) 10 μM epinephrine. The inhibition of cyclase caused by epinephrine in the upper panel is illustrated as the percentage of inhibition in the lower panel.
concentration exceeded 30 mM. Such Na⁺ requirement for epinephrine inhibition was not altered by IAP treatment of islets; no inhibition was observed in the membrane of IAP-treated islets as well unless the Na⁺ concentration was higher than 3 mM, though the degree of inhibition at higher Na⁺ concentrations was diminished by IAP treatment (Fig. 7B).

Comparison of the Action of Cholera Toxin with IAP Action—Islets were cultured with cholera toxin, and the membrane fraction prepared therefrom was assayed for adenylate cyclase activity with ATP as substrate (Fig. 8). In contrast to the IAP treatment, the cholera toxin treatment of islets caused a 5- to 6-fold increase in the basal activity of adenylate cyclase that was measured in the absence of activators such as guanine nucleotides (compare the values obtained without guanine nucleotide between Figs. 3 and 8). The addition of GTP to the toxin-treated preparation caused further increases in adenylate cyclase activity in a dose-dependent manner; this GTP-induced activation was markedly suppressed by simultaneously added epinephrine (Fig. 8A) again dependently on concentrations of GTP (Fig. 8C). In contrast, Gpp(NH)p did not activate the enzyme in the toxin-treated membrane. No inhibition was provoked by epinephrine in the presence of Gpp(NH)p. Marked inhibition, rather than activation, was produced by NaF of adenylate cyclase in the cholera toxin-treated membrane, and epinephrine was no longer inhibitory in the presence of fluoride. These effects of GTP, Gpp(NH)p, NaF, and epinephrine were reproduced in the membrane fraction that was prepared from islets cultured with cholera toxin plus IAP with the only exception that the degree of epinephrine inhibition in the presence of GTP was markedly diminished by superimposed IAP (Fig. 8, B and D). Thus, these two bacterial toxins, IAP and cholera, exert their own unique influence on the islet cell membrane in manners radically distinct from each other.

Effect of IAP Treatment on Receptor-mediated Activation of Adenylate Cyclase—Based on the results obtained with intact cell preparations, we have argued that receptor-mediated activation of adenylate cyclase would be enhanced by IAP treatment (5, 6, 21). Although agonist-induced activation of adenylate cyclase was only small in magnitude in our cell-free membrane preparations, the activation was, in fact, somewhat potentiated by prior treatment with IAP. For example, glucagon activated membrane adenylate cyclase in a dose-dependent manner; 20% (at 1 μM glucagon) and 40% (at 10 μM) stimulation was observed in the presence of 10 μM GTP, and 55% (at 1 μM) and 91% (at 10 μM) stimulation was observed in its absence. These values were increased to 35, 55, 77, and 125, respectively, in IAP-treated cell membranes.

Fig. 9 shows the effect of adenosine on membrane adenylate cyclase activity. In either the presence or absence of GTP, adenylate cyclase was slightly activated by adenosine from 1–10 μM but was conversely inhibited at 100 μM. The activation caused by lower concentrations is due to stimulation of R sites, since it was no longer observable unless Ro-20-1724 was replaced for a methylxanthine which is a specific antagonist of R sites (22, 23). This R site-mediated activation was more marked in membrane preparations from IAP-treated islets than in preparations from nontreated islets (Fig. 9). In contrast, the inhibition of the enzyme caused by higher concentrations (~100 μM) of adenosine, which is mediated by methylxanthine-insensitive P sites, was not affected by IAP pretreatment.

Discussion

Several insulin secretagogues cause cAMP accumulation in islet cells when they accelerate insulin discharge from the cells. Probably, these secretagogues stimulate their own receptors which, located on the membrane, are coupled through the mediation of the GTP-regulatory protein to the catalytic subunit of adenylate cyclase. Pretreatment of islets with IAP enhanced their insulin secretory responses to these receptor agonists; the enhanced secretion was mostly, if not totally, accounted for in terms of enhanced generation of cAMP within the islet cells (6, 21). Likewise, IAP reversal of an adrenergic inhibition of insulin secretion was invariably associated with restoration of cellular cAMP generation that should have been otherwise inhibited via α-adrenergic receptors (5, 6, 21). There was no difference, however, in the cellular cAMP content between IAP-treated and nontreated islets unless generation of the nucleotide was stimulated by the addition of receptor agonists (6, 21). Based on these findings, we have already argued (5, 6, 21) that receptor-mediated regulation of islet adenylate cyclase would be profoundly affected by the IAP treatment of islet cells. The present results have provided direct evidence for this argument; there was marked difference, under certain conditions, in the response of membrane adenylate cyclase between IAP-treated and nontreated islet cells.

The islet adenylate cyclase was activated by GTP, Gpp(NH)p, a GTP analog with a nonhydrolyzable terminal phosphate bond, NaF, and cholera toxin; all of these effectors are known to interact directly with the GTP-regulatory protein (see references 24 and 25 for recent review). GDP was also an activator of islet adenylate cyclase, but the degree of the activation was much smaller than that caused by GTP or Gpp(NH)p (Fig. 4). GDP competed with Gpp(NH)p (Fig. 6) which, in turn, provided a condition unfavorable for the oc-

![Figure 9: Effects of adenosine on adenylate cyclase.](image-url)
currence of GTP-mediated activation (Fig. 5). These findings would be accounted for by a currently accepted mechanism for the regulation of adenylate cyclase activity, i.e., binding of GTP or its nonhydrolyzable analog to the GTP-regulatory protein favors transition of the enzyme system to its active state while generation of the GDP-protein complex due to GTPase activity of the protein or displacement of GTP (or Gpp(NH)p) with GDP affords an inactive state of the enzyme (24–28). It should be emphasized here that this guanine nucleotide-dependent regulation of adenylate cyclase was not affected by IAP treatment. This, together with failure of IAP to alter the basal enzyme activity (estimated without addition of any ligand to the GTP-regulatory protein) and K_m for ATP or App(NH)p, makes it very likely that neither the catalytic unit nor the GTP-regulatory component in the adenylate cyclase complex is the site of IAP action.

Catalytic and regulatory properties of adenylate cyclase in IAP-treated islets were dissimilar to those in cholera toxin-treated islets. Cholera toxin treatment gave rise to a marked increase in basal activity of islet adenylate cyclase, which was not further activated by Gpp(NH)p, as had been reported for the enzymes from the toxin-treated rat liver (29) or cultured hamster ovary cells (30). NaF is well known to become inhibitory to adenylate cyclase after the toxin treatment (31). In sharp contrast, IAP was without effect on adenylate cyclase in these respects; the basal activity as well as its response to GTP, Gpp(NH)p, or NaF was not altered at all by the prior treatment with IAP of the enzyme donor islet cells. On the other hand, α-adrenergic inhibition of adenylate cyclase was attenuated after IAP treatment but was never altered after cholera toxin treatment of islets. Moreover, exposure of islets to both IAP and cholera toxin in combination resulted in alteration of enzymic properties which was to be induced independently by the exposure to either alone. These results may afford convincing evidence for the site and manner of IAP action to be distinct from those of cholera toxin and confirm the aforementioned conclusion that IAP does not interact with the GTP-regulatory protein in the adenylate cyclase holoenzyme.

Epinephrine inhibition of islet membrane adenylate cyclase may reflect a tight linkage of the α-receptor protein with the GTP-regulatory protein in the adenylate cyclase complex used in the present study. Since the α-adrenergic inhibition is readily susceptible to IAP action, it deserves further discussion as follows. In addition to α-adrenergic agents, several other receptor agonists are known to inhibit, rather than stimulate, adenylate cyclase in mammalian tissues. The inhibition of islet adenylate cyclase observed here is characterized by the findings that (a) it is strictly dependent on the addition of GTP, (b) the presence of Na^+ is required, and (c) epinephrine is similarly inhibitory to adenylate cyclase of islets pretreated with cholera toxin. These characteristics offer striking resemblances to those recently observed for the inhibition of adenylate cyclase in human platelets by α-agonists (32–34), in adipocytes by α-agonists (35, 36), by adenosine (23), by prostaglandin E_2 (36), and by nicotinic acid (36, 37), in hybrid cells (neuroblastoma x glioma) by α-agonists (38), by opiates (39), and by cholinergic agents (40), and in heart cells by cholinergic agents (41). For example, requirements for GTP (23, 32–41) and for Na^+ (35–37, 39–41) have been claimed, and inhibition was observed in the cell membranes treated with active fragments of cholera toxin as well (32, 41).

GTP requirement of this receptor-mediated inhibition of adenylate cyclase has been a clue to elucidation of the underlying mechanism. In the case of the adipocyte adenylate cyclase, GTP was inhibitory by itself at its higher concentra-

**Figure 10.** Correlation of the degree of epinephrine inhibition with GTP-induced activation of adenylate cyclase. The data in Figs. 3, 4, and 8 are used. The percentage of inhibition caused by epinephrine is plotted against the GTP-induced increment of adenylate cyclase (the increment over the "basal" value is expressed as the percentage of the maximal increment caused by the highest (0.1 mm) concentration of GTP). Data was obtained without (O, C) or with (A, △) cholera toxin treatment and without (O, △) or with (A, △) IAP treatment.
rine-induced inhibition without altering above mentioned characteristics of the inhibition; GTP and Na+ requirements of the inhibition were observed after IAP treatment as well. The affinity of epinephrine for α-adrenergic receptors as revealed by its concentration required for the half-maximal inhibition was not changed by IAP treatment (Fig. 2), making it unlikely that IAP directly interacts with the receptor protein. As shown in Fig. 10, epinephrine inhibition was well correlated with GTP activation of the enzyme in IAP-treated membranes (r = 0.924, p < 0.001). The correlation was not influenced by cholera toxin. The slope of the two plots in Fig. 10 was 0.22 ± 0.019 and 0.46 ± 0.026 for the IAP-treated and nontreated membrane, respectively. These two slope values are significantly different from each other (p < 0.001), clearly indicating that IAP treatment diminished the magnitude of epinephrine inhibition without altering GTP dependence of the inhibition. The competition between Gpp(NH)p and GDP was retained in the IAP-treated enzyme (Fig. 6). Conceivably, IAP treatment would result in a certain modification of the mechanism functionally connecting the receptor protein with the GTP-regulatory protein without directly affecting these two components.

Adenosine exerted biphasic effects on islet adenylate cyclase, activation at its lower concentrations and inhibition at higher concentrations (Fig. 9). Similar results were obtained with the platelet enzyme (43). Probably R sites mediate the activation while P sites are responsible for the inhibition since the activation was interfered with, but the inhibition was not, by a methylxanthine, an R site antagonist (22, 23). It has been argued that R sites behave like other membrane receptors for hormones or neurotransmitters (22) whereas P sites are located on the intracellular membrane and inseparable from the catalytic protein of adenylate cyclase (44). P site-mediated inhibition of adenylate cyclase, unlike α-adrenergic inhibition, was not affected by IAP treatment, in support of the foregoing discussion that the site of IAP action was not the catalytic unit of the membrane enzyme system. Instead, R site-mediated activation of adenylate cyclase, like glucagon activation, was slightly enhanced by IAP treatment. Such enhancement, together with attenuation of α-adrenergic inhibition, led us to a conclusion that the action of IAP is directed to the mechanism regulating the receptor-mediated regulation of adenylate cyclase.

It has been recently shown that IAP is capable of interaction with rat heart cells as well; an accumulation of cAMP during incubation of heart cells with a β-adrenergic agent or glucagon was exaggerated, and its inhibition via muscarinic cholinergic receptors was abolished by prior treatment of the cells with IAP (45). Both R and P adenosine sites also mediated the inhibition of the cAMP accumulation in heart cells; the inhibition mediated by R sites was, but the inhibition via P sites was not, reversed by IAP pretreatment (45). Thus, receptor-mediated regulation of adenylate cyclase is very likely to be susceptible to IAP-induced modification even in heart cells and possibly in other cells. It is tempting to speculate that IAP interacts with a putative coupling factor that is implied to be tightly bound to the GTP-regulatory protein and lacking in the uncoupled variant of the S49 lymphoma cell (46). In any case, IAP will be a very promising probe, in further studies, for providing real insights into the molecular mechanism of interaction between the membrane receptors and adenylate cyclase.

REFERENCES

1. Yajima, M., Hosoda, K., Kanbayashi, Y., Nakamura, T., Nogimori, K., Nakao, Y., and Ui, M. (1978) J. Biochem. (Tokyo) 83, 295-303
2. Yajima, M., Hosoda, K., Kanbayashi, Y., Nakamura, T., Takahashi, I., and Ui, M. (1978) J. Biochem. (Tokyo) 83, 305-312
3. Ui, M., Katada, T., and Yajima, M. (1978) in International Symposium on Pertussis (Manclark, C. R., and Hill, J. C., eds) Department of Health, Education, and Welfare Publication 79-180, pp. 166-173, National Institute of Health, Bethesda, Maryland
4. Munoz, J. J., and Bergman, R. K. (1978) in International Symposium on Pertussis (Manclark, C. R., and Hill, J. C., eds) Department of Health, Education, and Welfare Publication 79-180, pp. 143-150, National Institute of Health, Bethesda, Maryland
5. Katada, T., and Ui, M. (1980) J. Biol. Chem. 255, 9580-9588
6. Katada, T., and Ui, M. (1979) J. Biol. Chem. 254, 469-479
7. Katada, T., and Ui, M. (1979) Endocrinology 104, 1822-1827
8. Lacy, P. E., and Kostianovsky, M. (1967) Diabetes 16, 35-39
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
10. Honma, M., Sato, T., Takekawa, J., and Ui, M. (1977) Biochem. Med. 18, 257-273
11. Chan, F. S., and Lin, M. C. (1974) Methods Enzymol. 48, 38-41
12. Kuo, W.-N., Hodgson, D. S., and Kuo, J. F. (1978) J. Biol. Chem. 248, 2705-2711
13. Howell, S. L., and Montague, W. (1973) Biochin. Biophys. Acta 320, 44-52
14. Johnson, D. G., Thompson, W. J., and Williams, R. H. (1974) Biochemistry 13, 1920-1924
15. Ismaili, N. A., and Montague, W. (1977) Biochin. Biophys. Acta 498, 325-330
16. Ismaili, N. A., El Denshary, E.-E. S. M., and Montague, W. (1977) Biochem. J. 164, 409-413
17. Davis, B., and Lazarus, N. R. (1972) Biochem. J. 129, 373-379
18. Goldfine, I. D., Roth, J., and Birnbaumer, L. (1972) J. Biol. Chem. 247, 1211-1218
19. Cassel, D., Levkovitz, H., and Selinger, Z. (1977) J. Cyclic Nucleotide Res. 3, 393-406
20. Cassel, D., Eckstein, F., Lowe, M., and Selinger, Z. (1979) J. Biol. Chem. 254, 9835-9838
21. Katada, T., and Ui, M. (1981) J. Biochem. (Tokyo) 89, 979-990
22. Londos, C., and Wolf, J. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5482-5486
23. Londos, C., Cooper, D. M. F., Schlegel, W., and Rodbell, M. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 5362-5366
24. Rodbell, M. (1980) Nature 284, 17-22
25. Abramowitz, J., Iyengar, R., and Birnbaumer, L. (1979) Mol. Cell. Endocr. 16, 129-146
26. Birnbaumer, L., Swartz, T. L., Abramowitz, J., Mintz, P. W., and Iyengar, R. (1980) J. Biol. Chem. 255, 3542-3551
27. Birnbaumer, L., Bearer, C. F., and Iyengar, R. (1980) J. Biol. Chem. 255, 9580-9588
28. Kasel, D., and Selinger, Z. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 4155-4159
29. Flores, J., and Sharp, G. W. G. (1975) J. Clin. Invest. 56, 1345-1349
30. Evin, D., and Anderson, W. B. (1979) J. Biol. Chem. 254, 8726-8729
31. Gill, D. M. (1977) Adv. Cyclic Nucleotide Res. 8, 85-118
32. Jakobs, K. H., and Schultz, G. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 310, 121-127
33. Steer, M. L., and Wood, A. (1979) J. Biol. Chem. 254, 10791-10797
34. Jakobs, K. H., Saur, W., and Schultz, G. (1978) FEBS Lett. 85, 167-170
35. Aktories, K., Schultz, G., and Jakobs, K. H. (1979) FEBS Lett. 107, 100-104
36. Aktories, K., Schultz, G., and Jakobs, K. H. (1980) Naunyn-Schmiedeberg's Arch. Pharmacol. 312, 167-173
37. Aktories, K., Jakobs, K. H., and Schultz, G. (1980) FEBS Lett. 115, 11-14
38. Sabol, S. L., and Nirenberg, M. (1979) J. Biol. Chem. 254, 1513-1520
39. Blume, A. J., Lichtshtein, D., and Boone, C. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 5626-5630
40. Lichtshtein, D., Boone, G., and Blume, A. (1979) J. Cyclic Nucleotide Res. 5, 367-375
41. Jakobs, K. H., Aktories, K., and Schultz, G. (1979) Naunyn-
Control of Adenylate Cyclase by Islet-activating Protein

Schmiedeberg’s Arch. Pharmacol. 310, 113–119
42. Cooper, D. M. F., Schlegel, W., Lin, M. C., and Rodbell, M. (1979) J. Biol. Chem. 254, 8927–8931
43. Jakobs, K. H., Saur, W., and Johnson, R. A. (1979) Biochim. Biophys. Acta 583, 409–421
44. Premont, J., Guillou, G., and Bockaert, J. (1979) Biochem. Biophys. Res. Commun. 90, 513–519
45. Hazeki, O., and Ui, M. (1981) J. Biol. Chem. 256, 2856–2862
46. Sternweis, P. C., and Gilman, A. G. (1979) J. Biol. Chem. 254, 3333–3340