Homologous desensitization of β -adrenergic receptors in lymphoma cells is not altered by the inactivation of N_i (G_i), the inhibitory guanine nucleotide regulatory protein

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We had previously demonstrated that the cyc^- mutant of S49 wild-type lymphoma cells both desensitizes and undergoes a sequestration–internalization of the β -receptor in response to short-term treatment with adrenaline. The cyc^- mutant of S49 wild-type lymphoma cells lacks the α_s subunit of the stimulatory coupling protein N_s , but has fully functional N_i , the inhibitory component of the regulatory complex. This suggested that functional N_s was not required for desensitization. To examine the role of N_i in desensitization, both S49 wild-type and cyc^- cells were treated with islet-activating protein under conditions that led to over 85% attenuation of N_i function in S49 wild-type cells and approx. 50% attenuation of N_i function in cyc^- cells. This treatment had no effect on the adrenaline-induced desensitization of adenylate cyclase or the sequestration event measured by the apparent movement of β -adrenergic receptors to a light-vesicle fraction. Further, the desensitization event, which occurs before the sequestration event, observable only in intact cells, was also not altered by islet-activating-protein pretreatment of S49 wild-type cells. The data suggest that a functional N_i is not required for desensitization in the S49 lymphoma cells.

INTRODUCTION

Our demonstration that adrenaline desensitized the cyc^- variant of S49 wild-type lymphoma cells led to the conclusion that neither functional stimulatory coupling proteins nor increased cyclic AMP was required for homologous desensitization (Green & Clark, 1981; Green *et al.*, 1981). More recently it has been shown that sequestration of the β -receptor also occurs in cyc^- cells, as measured by an adrenaline-induced shift in cyc^- -cell β -receptors to a light-vesicle fraction (Clark *et al.*, 1985a), and by the isoprenaline-induced loss of surface receptors on intact cyc^- cells (Staehelin & Hertel, 1983; Mahan *et al.*, 1985).

The recent discoveries (i) that cyc^- cells have a functional inhibitory coupling protein (N_i) (Hildebrandt *et al.*, 1982; Jakobs *et al.*, 1983), (ii) that the $\beta\gamma$ subunit of N_i from erythrocytes and rabbit liver is probably identical with the $\beta\gamma$ subunit of N_s (Codina *et al.*, 1984; Manning & Gilman, 1983), and (iii) that the purified turkey erythrocyte β -adrenergic receptor is capable of stimulating purified rabbit liver N_i GTPase activity in reconstituted vesicles, led to the suggestion by Asano *et al.* (1984) that either N_i or the $\beta\gamma$ subunits may be involved in desensitization. Preliminary results from several laboratories have suggested that N_i may play a role in glucagon-induced desensitization of hepatocytes (Heyworth *et al.*, 1984) and the MDCK cell line (Rich *et al.*, 1984). The glucagon-induced heterogeneous desensitization of the MDCK line results in an increased ADP-ribosylation of N_i in membranes, whereas in hepatocytes the glucagon-induced desensitization is

blocked or obscured by islet-activating protein (IAP) pretreatment, suggesting a role for N_i in glucagon desensitization. It was not clear from either of these studies whether N_i was involved in homologous desensitization.

In the present experiments we have examined the role of functional N_i in the homologous β -adrenergic-agonist desensitization of wild-type and cyc^- lymphoma cells by comparing desensitization in cells pretreated with or without IAP. Ui and collaborators have shown that pretreatment of various cells with IAP, a purified toxin from *Bordetella pertussis*, leads to the loss of N_i function through the ADP-ribosylation of the α_i subunit (Ui *et al.*, 1984; Katada & Ui, 1982). Several groups have shown that both the wild-type and cyc^- S49 lymphoma cells lose N_i function after IAP treatment (Katada *et al.*, 1984; Jakobs *et al.*, 1983). We found that neither the extent of desensitization of adrenaline-stimulated adenylate cyclase nor the sequestration of the β -adrenergic receptor appears to be altered by IAP, supporting and extending the previous conclusion that functional coupling proteins are not required or involved in homologous desensitization of lymphoma cells. A preliminary report of these results has been published (Clark *et al.*, 1985b).

EXPERIMENTAL

Pretreatment, cell lysis and membrane fractionation

Wild-type or cyc^- cells were pretreated for 16 h at 37 °C with or without IAP at a final concentration of 10 ng/ml. IAP was kindly provided by Dr. Michio Ui and Dr.

Abbreviations used: IAP, islet-activating protein; N_i , inhibitory guanine nucleotide coupling protein; N_s , stimulatory guanine nucleotide coupling protein; p[NH]ppG, guanosine 5'-[β , γ -imido]triphosphate; IPIN, [125 I]iodopindolol.

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Toshiaki Katada, Hokkaido University, Sapporo 060, Japan. IAP was stored in 0.1 M-K₂HPO₄/KH₂PO₄, pH 7.4, with 2 M-urea, and controls received the appropriate dilution of this buffer (Katada & Ui, 1982). After IAP pretreatment, the cells were collected and suspended at a density of 30×10^4 – 40×10^6 cells/ml in serum-free medium containing 20 mM-Hepes, pH 7.4. Cells were then treated at 37 °C for 30 min with 1 μ M-adrenaline in 0.1 mM-ascorbate and 1.0 mM-thiourea, or with only the ascorbate/thiourea. After the hormone treatment, the cells were rapidly cooled to 0–4 °C and collected by centrifugation at 600 *g* for 5 min. The cells were then washed, and lysed, and the 600 *g* supernatant was fractionated on sucrose gradients (19–45%, w/w) as previously described (Clark *et al.*, 1985a).

Measurement of β -adrenergic receptors

The distribution of β -receptors in the various sucrose-gradient fractions was determined by [¹²⁵I]iodopindolol (IPIN) binding. IPIN was prepared by iodination of l-pindolol as described previously (Barovsky & Brooker, 1980). Samples (50 μ l) of the gradient fractions were incubated with approx. 500 pM-IPIN in 10 mM-Tris/HCl (pH 7.5)/5 mM-MgCl₂ for 30 min at 30 °C in a final volume of 100 μ l. The incubation was stopped by the addition of 3 ml of ice-cold buffer followed by filtration on Whatman GF/C filters.

Adenylate cyclase assay

This was measured by the procedure of Salomon *et al.* (1984). The assay solution comprised 40 mM-Hepes, pH 7.7, 2 mM or 6 mM-MgCl₂, 0.2 mM-ATP, 1 mM-EDTA, 8 mM-phosphocreatine, 16 units of creatine kinase/ml, 0.1 mM-3-isobutyl-1-methylxanthine, 2–3 μ Ci of [α -³²P]ATP, and 30 μ l of the appropriate heavy-vesicle fraction in a final volume of 100 μ l: 2 mM-MgCl₂ was used to assess the effect of IAP on inhibitions by p[NH]ppG and somatostatin, and 6 mM-MgCl₂ was used for all other assays. Incubations were carried out for 10 min at 30 °C.

Reconstitution of *cyc*[−]-cell membranes

Heavy vesicle fractions from *cyc*[−] cells (fractions 18–22) were reconstituted by a modification of the Sternweis & Gilman (1979) procedure as previously described (Clark *et al.*, 1984a). The reconstituted samples were then assayed for adrenaline-stimulated adenylate cyclase activity.

Intact-cell studies

The effect of adrenaline on intact cells was determined by the prelabelling techniques previously described (Butcher, 1978). The ATP pool was prelabelled with [2,8-³H]adenine, and the percentage conversion of [³H]AXP into cyclic [³H]AMP was measured ([³H]AXP = [³H]ATP + [³H]ADP + cyclic [³H]AMP).

ADP-ribosylation of N_i

Heavy-vesicle fractions (200 μ g) were incubated in 200 μ l of a solution containing 10 μ M-NAD⁺, 1 mM-ATP, 0.2 mM-GTP, 5 mM-MgCl₂, 10 mM-thymidine, 10 mM-arginine, 5 mM-phosphocreatine, 16 units of creatine kinase/ml, 1 mM-EDTA, 20 mM-Tris/HCl, pH 7.4, 1–5 μ Ci of [³²P]NAD⁺ and 5 μ g of IAP or its vehicle. [³²P]NAD⁺ was provided by Dr. Lutz Birnbaumer and Dr. Ravi Iyengar, Baylor Medical School, Houston, TX,

U.S.A. Incubations were at 30 °C for 30 min and were terminated by the addition of ice-cold 20 mM-Tris/HCl, pH 7.4. These conditions gave optimum ADP-ribosylation of N_i and were basically those described by Katada & Ui (1982). The membranes were centrifuged at 36000 *g* for 15 min and washed once in 20 mM-Tris buffer. The pellets were suspended in 100 μ l of buffer containing 10% (v/v) glycerol, 5% (v/v) β -mercaptoethanol, 3% (w/v) SDS and 62.5 mM-Tris/HCl, pH 6.8. Polyacrylamide-gel electrophoresis was performed by the procedure of Laemmli (1970), with the following protein standards: trypsinogen, fumarase, catalase, bovine serum albumin and human transferrin (from Sigma).

RESULTS

IAP pretreatment attenuates N_i function and cell-free ADP-ribosylation

The standard IAP pretreatment protocol of wild-type cells used in all of the subsequent experiments resulted in over 85% attenuation of inhibitions by somatostatin and p[NH]ppG of forskolin-stimulated adenylate cyclase (Fig. 1a). Similar treatment of *cyc*[−] cells resulted in an approx. 40–70% attenuation of inhibitions by p[NH]ppG and somatostatin of adenylate cyclase (Fig. 1b). These

Fig. 1. Attenuation of inhibition by p[NH]ppG and somatostatin of forskolin-stimulated adenylate cyclase after IAP pretreatment of wild-type and *cyc*[−] cells

Heavy-peak sucrose-gradient fractions from wild-type (a) and *cyc*[−] (b) cells which had been pretreated with (○) or without (●) 10 ng of IAP/ml for 16 h were combined and assayed for 50 μ M-forskolin-stimulated adenylate cyclase activity with various concentrations of p[NH]ppG and somatostatin. Values shown are percentages of forskolin-stimulated activity in the absence of inhibitors, and represent the means $\pm$ S.E.M. of three experiments.

Fig. 2. ADP-ribosylation of heavy-vesicle fractions from wild-type and cyc^- cells pretreated with or without IAP

Sucrose-gradient heavy-vesicle fractions from cells that had been pretreated (I) with or without 10 ng of IAP/ml for 16 h were combined, washed with 20 mM-Hepes/1 mM-EDTA, pH 8, and suspended in that medium. The vesicles were incubated (II) with [32 P]NAD $^+$ with or without IAP as described and run on SDS/polyacrylamide-gel electrophoresis. The resulting autoradiograms are shown for cyc^- (a) and wild-type (b) cells. The heavily labelled bands at the bottom of the gel in (a) were at the dye front. In the experiments with wild-type cells (b), the dye front migrated off the gel. The lanes shown above were as follows.

Cells	Lane	Incubation I (16 h, 10 ng of IAP/ml)	Incubation II ADP-ribosylation (5 μ g of IAP)
cyc^-	1	—	—
	2	—	IAP
	3	IAP	—
	4	IAP	IAP
Wild-type	1	IAP	—
	2	IAP	IAP
	3	—	—
	4	—	IAP

results are in agreement with the earlier work by Katada *et al.* (1984). The IAP treatment of wild-type cells usually, although not always, resulted in a decrease in forskolin-stimulated adenylate cyclase with the low- Mg^{2+} conditions (~ 0.75 mM free Mg^{2+}) used in these assays of adenylate cyclase. In contrast, IAP treatment consistently resulted in increased adrenaline stimulation of adenylate cyclase activity and cyclic AMP accumulation in both wild-type and cyc^- cells (see Figs. 4 and 5 and Tables 1 and 2). However, the effect of IAP on forskolin and adrenaline stimulation is markedly dependent on temperature and the Mg^{2+} concentration (M. A. Proll, R. W. Butcher, R. B. Clark & R. Barber, unpublished work). Forskolin-stimulated activity of cyc^- -cell adenylate cyclase was not significantly different in control or IAP-pretreated membranes with the low- Mg^{2+} conditions.

To assess further the extent of IAP action under standard conditions, membranes from both cyc^- and wild-type cells were ADP-ribosylated in the presence of IAP and [32 P]NAD $^+$ (Fig. 2). The extent of ADP-

ribosylation of N_i was determined by SDS/polyacrylamide-gel electrophoresis and autoradiography. IAP-pretreated membranes showed less than 5–10% of the [32 P]ADP-ribosylation of N_i compared with control membranes; in fact, ADP-ribosylation of N_i in the IAP-pretreated membranes was evident only with prolonged autoradiography.

Lack of an effect of IAP pretreatment of wild-type and cyc^- cells on the adrenaline-induced desensitization of β -receptors

We have previously demonstrated (Clark *et al.*, 1985a) that adrenaline treatment of both wild-type and cyc^- cells induces a redistribution of β -receptors on sucrose gradients, i.e. an increased number of receptors in the light-vesicle fractions, which appears to be attributable to sequestration and/or internalization. In addition, this treatment leads to a 3–4-fold increase in the $K_{act.}$ of adrenaline stimulation of the heavy-vesicle adenylate cyclase and a decrease in the $V_{max.}$ In the present experiments both wild-type and cyc^- cells were pretreated

Fig. 3. Adrenaline-induced redistribution of β -adrenergic receptors on sucrose gradients from wild-type and cyc^- cells pretreated with or without IAP

cyc^- (a) and wild-type (b) cells were treated overnight at 37 °C with (○, △) or without (●, ▲) 10 ng of IAP/ml. After 16 h, the cells were centrifuged, suspended in serum-free Hepes-buffered medium, and treated for 30 min with (△, ▲) or without (○, ●) 1 μ M-adrenaline. The cells were then cooled, washed and lysed, and the 600 g supernatant fraction was centrifuged on sucrose gradients. β -Receptors in the gradient fractions were assayed by IPIN binding (500 pM).

with or without IAP, then incubated with 1.0 μ M-adrenaline for 30 min. The 600 g supernatants from the lysates were then layered over sucrose gradients and the distribution of the β -receptors was measured with IPIN. In both wild-type and cyc^- cells, adrenaline induced the redistribution of approx. 25–30% of the receptors into light vesicles (Figs. 3a and 3b). The IAP pretreatment had no effect on either the redistribution or the total number of β -receptors.

To determine whether or not the adenylate cyclase of wild-type cells was desensitized after the various pretreatments, heavy-peak sucrose-gradient fractions 18–22 were collected and assayed over a range of adrenaline concentrations (Fig. 4). Eadie-Hofstee transformations of the data revealed that the typical 3-fold adrenaline-induced increase in $K_{act.}$ and decrease in $V_{max.}$ was not altered by IAP pretreatment. A summary of three experiments identical with that in Fig. 4 is given in Table 1, showing that IAP increased the $V_{max.}$ for adrenaline stimulation of adenylate cyclase 1.6-fold either with or without the subsequent adrenaline-induced desensitization.

In cyc^- cells, similar qualitative results were obtained (Table 2) with adrenaline-stimulated adenylate cyclase activity after reconstitution of cyc^- -cell heavy-vesicle fractions with cholate extracts of wild-type cells (heat-killed to destroy catalytic activity in the extract). The adrenaline-induced desensitization was unaffected by IAP, and there was a slight, although not significant increase in adrenaline stimulation after the IAP pretreatment.

Effect of IAP on adrenaline-stimulated cyclic AMP accumulation in intact wild-type cells

We have previously found that adrenaline-induced desensitization of intact S49 wild-type cells occurs at low

concentrations, where little if any desensitization is observed in subsequent cell-free assay (Clark *et al.*, 1985a; Barber *et al.*, 1984). To determine whether or not the overnight IAP pretreatment altered the adrenaline-induced desensitization at low concentrations of adrenaline, the accumulation of cyclic AMP in intact wild-type

Fig. 4. Adrenaline-induced desensitization of wild-type cells pretreated with or without IAP

Wild-type cells were pretreated with (○, △) or without (●, ▲) IAP (10 ng/ml for 16 h) and subsequently desensitized with (△, ▲) or without (●, ○) 1 mM-adrenaline for 30 min. Heavy-peak fractions from sucrose gradients were assayed for adrenaline-stimulated adenylate cyclase activity (v) with hormone concentrations of 0.01–1.0 μ M. The Eadie-Hofstee transformation of the activities is presented. (Basal activity was subtracted from all values.) The data are representative of three similar experiments.

Table 1. Effect of IAP on desensitization of wild-type cells

Wild-type cells were pretreated with or without 10 ng of IAP/ml for 16 h (Incubation I), then desensitized with 1.0 μ M-adrenaline for 30 min (Incubation II). Cells were then cooled, washed, lysed and run on sucrose gradients as described in the Experimental section. Adenylate cyclase activity was measured at various adrenaline concentrations as described in Fig. 4 legend. $V_{\max}$ and K_{act} were derived from Eadie-Hofstee plots of the data. Values shown are the means $\pm$ S.E.M. from three experiments.

Incubation I (16 h)	Incubation II (30 min)	$V_{\max}$ (pmol of cyclic AMP/ min per mg)	Decrease in $V_{\max}$ (%)	K_{act} (nM)	Adrenaline-induced increase in K_{act} (fold)
Control	– Adrenaline	825 $\pm$ 173	—	32.4 $\pm$ 2.9	—
	+ Adrenaline	507 $\pm$ 101	39	99.2 $\pm$ 8.1	3.0
IAP (10 ng/ml)	– Adrenaline	1333 $\pm$ 163	—	35.7 $\pm$ 2.3	—
	+ Adrenaline	807 $\pm$ 109	39	109 $\pm$ 13.5	3.0

Table 2. Effect of IAP on adrenaline-induced desensitization of Cyc^- cells

Heavy-vesicle fractions 18–22 from cyc^- cells, which had been pretreated with or without IAP and subsequently treated for 30 min with or without 1 μ M-adrenaline, were reconstituted with crude heat-killed cholera extracts of N_i from wild-type cells. The reconstituted membranes were assayed for basal and adrenaline-stimulated adenylate cyclase activities. The values shown are the means $\pm$ one-half the range from two experiments: values in parentheses are the percentage desensitization.

Incubation I (16 h)	Incubation II (30 min)	Adenylate cyclase (pmol of cyclic AMP/min per mg)		
		Basal	+ 25 nM-Adrenaline	+ 10 mM-Adrenaline
Control	– Adrenaline	1.9 $\pm$ 0.6	10.3 $\pm$ 1.3	36.6 $\pm$ 2.8
	+ Adrenaline	2.4 $\pm$ 0.8	6.8 $\pm$ 0.3 (47)	29.7 $\pm$ 4.4 (21)
IAP (10 ng/ml)	– Adrenaline	3.1 $\pm$ 0.7	13.8 $\pm$ 3.5	44.8 $\pm$ 5.0
	+ Adrenaline	2.9 $\pm$ 0.7	8.5 $\pm$ 0.7 (48)	34.5 $\pm$ 1.2 (24)

cells was followed as a function of the time of treatment with 20 nM-adrenaline (Fig. 5). Within 2 min cyclic AMP concentrations had reached a maximum, after which they decayed to near-basal values. The shape of the curves was unaltered by IAP, as best shown in the normalized data (inset to Fig. 5), although, as in the cell-free measurements, the IAP pretreatment did lead to a greater adrenaline stimulation of adenylate cyclase. It has been previously shown by Barber & Butcher (1982) that the decrease in cyclic AMP in intact wild-type cells from peak values is not due to activation of phosphodiesterase, but rather to desensitization. This was determined by two separate procedures: the double-label procedure and the measurement of the decrease in cyclic AMP after addition of propranolol. In the present experiments we found that the rate of the decrease in cyclic AMP after addition of propranolol was unaffected by IAP (results not shown).

DISCUSSION

In wild-type cells, IAP induced the loss of over 85% of the somatostatin and p[NH]ppG inhibition of adenylate cyclase and eliminated ADP-ribosylation of N_i in membranes. In spite of the rather considerable attenuation of N_i function, adrenaline-induced desensitization of adenylate cyclase and sequestration of the β -receptor proceeded normally. Since desensitization of adenylate cyclase in cell-free assays and sequestration–internal-

ization are only observed after treatment of the wild-type cells with relatively high concentrations of adrenaline (> 50 nM), it was necessary to monitor the effect of IAP on the desensitization of wild-type cells at a low concentration of adrenaline (20 nM). The data (Fig. 5) indicate that, although IAP produced its characteristic augmentation of adrenaline-stimulated activity, desensitization was not affected. Our results suggest that a functional N_i is not required for the early rapid homologous adrenaline-induced desensitization and sequestration–internalization of the β -adrenergic receptor in the S49 wild-type lymphoma cells and the cyc^- variant.

The conclusion that functional N_i is not required for desensitization is supported by preliminary results from our laboratory, which indicate that homologous adrenaline-induced desensitization of WI-38 fibroblasts was also unaffected by IAP pretreatment. The IAP treatment was of sufficient duration to eliminate the muscarinic inhibition of cyclic AMP accumulation in these cells. Further, the heterologous prostaglandin E_1 -induced desensitization of these cells was also unaffected by IAP inactivation of N_i . Thus it appears evident that, although the extent of pertussis-toxin-catalysed ADP-ribosylation of N_i alters adrenaline stimulation of adenylate cyclase in several cell types, elimination of N_i function by ADP-ribosylation does not affect hormone-induced desensitization in lymphoma cells or fibroblasts. In other words, alterations of N_i function could be a mechanism

Fig. 5. Effect of IAP pretreatment on adrenaline-stimulated cyclic AMP contents in wild-type cells

Wild-type cells were pretreated for 16 h with (●) or without (○) 10 ng of IAP/ml. Subsequently, the cells were prelabelled with [3 H]adenine and incubated with 20 nM-adrenaline at 37 °C. Cyclic AMP contents, i.e. the percentage conversion of AXP into cyclic AMP, was measured at the times indicated on the abscissa. Values shown are the means of triplicate determinations. A portion of the cells after the IAP treatment was used for assessing the effect of IAP on inhibition by p[NH]ppG and somatostatin of adenylate cyclase as described in Fig. 1. The loss of inhibitory activity as a result of the IAP treatment, assessed by p[NH]ppG inhibition, in membranes from this experiment was over 90%. The inset shows the percentage conversion values normalized to the value at 2 min.

of sensitization or desensitization (sensitization if N_i activation was prevented; desensitization if N_i was activated), but they do not appear to be involved in either adrenaline-induced homologous desensitization of lymphoma cells and fibroblasts or prostaglandin E_1 -induced heterologous desensitization of fibroblasts. Since the glucagon desensitization of hepatocytes is altered by IAP pretreatment (Heyworth *et al.*, 1984), it seems likely that the mechanisms of desensitization of these cells by glucagon differ from those of the β -adrenergic receptor in lymphoma cells and fibroblasts. This is supported by the observations that short-term glucagon pretreatment leads to an increase in p[NH]ppG-stimulated adenylate cyclase activity (Heyworth & Houslay, 1983), whereas long-term treatment actually leads to heterologous desensitization; i.e. adrenaline-, NaF- and p[NH]ppG-stimulated activities are decreased (Noda *et al.*, 1984). Further preliminary evidence suggests that the glucagon desensitization does not involve internalization (Heyworth

& Houslay, 1983; Noda *et al.*, 1984). Adrenaline desensitization of hepatocytes, by contrast appears to be a typical homologous β -adrenergic-receptor desensitization (Noda *et al.*, 1984).

The capacity of IAP to block inhibitory activity in membranes from cyc^- cells was not as dramatic as in wild-type cells, for reasons not at present understood, even though the ADP-ribosylation of N_i in the membranes revealed that the effect of IAP pretreatment by this criterion was comparable with that in the wild-type cells. Obviously a comparison of the extent of p[NH]ppG inhibition of adenylate cyclase in membranes from cyc^- and wild-type cells is not straightforward. In wild-type cells, which are replete with N_s , it seems likely that N_s more effectively obscures the effects of residual N_i activation by p[NH]ppG after IAP inactivation. Of course, p[NH]ppG itself stimulates the wild-type cyclase, and for that reason the inhibitory effect of p[NH]ppG is seen only when forskolin concentrations exceed 10–20 μ M (R. B. Clark, unpublished work). In cyc^- cells, which have no detectable α_s , activation of even a small fraction of N_i may be sensed by the forskolin-activated adenylate cyclase.

Why IAP treatment leads to an increased response in adrenaline in both intact wild-type cells and cell-free assays is not understood. However, it is a general phenomenon, and it has been suggested that this may be the result of the loss of a β -receptor- N_i interaction (Itoh *et al.*, 1984). Abramson *et al.* (1985) have presented preliminary evidence that GTP decreases the affinity of direct agonist binding to S49 cyc^- -cell lymphoma membranes, using [3 H]hydroxybenzylisoprenaline. Since it appears that cyc^- cells lack a functional α_s , the GTP effect on agonist binding could reflect a β -receptor- N_i interaction. This is indirectly supported by the demonstration that turkey β -receptor stimulates N_i GTPase activity (Asano *et al.*, 1984). These data could explain the increase in adrenaline stimulation after IAP treatment of wild-type cells. However, it is equally plausible that it is due to the loss of a basal inhibitory activity of N_i ; that is, GTP, which must be present for N_s -mediated stimulation, activates N_i as well, and loss of this activation after IAP treatment would promote hormone stimulation. Further extrapolation from these results must await the direct assessment of the capacity of the β -receptor from lymphoma cells to interact with ADP-ribosylated N_i or the $\beta\gamma$ subunits. Also, since N_i may be in excess of the β -receptor, it is not yet clear whether a very small percentage of non-ADP-ribosylated N_i , although inadequate for mediating inhibition of adenylate cyclase, could be sufficient to mediate desensitization and internalization.

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REFERENCES

- Abramson, S. N., Reynolds, E. E., Hoyer, D. & Molinoff, P. B. (1985) Adv. Cyclic Nucleotide Protein Phosphorylation Res. **19**, 13–27.
- Asano, T., Katada, T., Gilman, A. G. & Ross, E. M. (1984) J. Biol. Chem. **259**, 9351–9354.

- Barber, R. & Butcher, R. W. (1982) in *Cell Regulation by Intracellular Signals* (Swillens, S. & Dumont, J. E., eds.), pp. 117–135, Plenum Press, New York
- Barber, R., Goka, T. J. & Butcher, R. W. (1984) *Mol. Cell. Endocrinol.* **36**, 29–35
- Barovsky, K. & Brooker, G. (1980) *J. Cyclic Nucleotide Res.* **6**, 297–307
- Butcher, R. W. (1978) *J. Cyclic Nucleotide Res.* **4**, 411–421
- Clark, R. B., Friedman, J., Prashad, N. & Ruoho, A. E. (1985a) *J. Cyclic Nucleotide Protein Phosphorylation Res.* **10**, 97–119
- Clark, R. B., Proll, M. A. & Friedman, J. (1985b) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44**, 1796
- Codina, J., Hildebrandt, J. D., Sekura, R. D., Birnbaumer, M., Bryan, J., Manclark, C. R., Iyengar, R. & Birnbaumer, L. (1984) *J. Biol. Chem.* **259**, 5871–5886
- Green, D. A. & Clark, R. B. (1981) *J. Biol. Chem.* **256**, 2105–2108
- Green, D. A., Friedman, J. & Clark, R. B. (1981) *J. Cyclic Nucleotide Res.* **7**, 161–172
- Heyworth, C. M. & Houslay, M. D. (1983) *Biochem. J.* **214**, 93–98
- Heyworth, C. M., Hanski, E. & Houslay, M. D. (1984) *Biochem. J.* **222**, 189–194
- Hildebrandt, J. D., Hanoune, J. & Birnbaumer, L. (1982) *J. Biol. Chem.* **257**, 14723–14725
- Itoh, H., Okajima, F. & Ui, M. (1984) *J. Biol. Chem.* **259**, 15464–15473
- Jakobs, K. H., Aktories, K. & Schultz, G. (1983) *Nature (London)* **303**, 177–178
- Katada, T. & Ui, M. (1982) *J. Biol. Chem.* **257**, 7210–7216
- Katada, T., Bokoch, G., Smigel, M. D., Ui, M. & Gilman, A. G. (1984) *J. Biol. Chem.* **259**, 3586–3595
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Mahan, L. C., Koachman, A. M. & Insel, P. A. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 129–133
- Manning, D. R. & Gilman, A. G. (1983) *J. Biol. Chem.* **258**, 7059–7063
- Noda, C., Shinjyo, F., Tomomura, A., Kato, S., Nakamura, T. & Ichihara, A. (1984) *J. Biol. Chem.* **259**, 7747–7754
- Rich, K. A., Codina, J., Floyd, G., Sekura, R., Hildebrandt, J. D. & Iyengar, R. (1984) *J. Biol. Chem.* **259**, 7893–7901
- Salomon, Y., Londos, C. & Rodbell, M. (1984) *Anal. Biochem.* **58**, 541–548
- Staehelin, M. & Hertel, C. (1983) *J. Recept. Res.* **3**, 35–43
- Sternweis, P. C. & Gilman, A. G. (1979) *J. Biol. Chem.* **254**, 3333–3340
- Ui, M., Katada, T., Murayama, T., Kurose, H., Yajuna, M., Tamura, M., Nakamura, T. & Nogimori, K. (1984) *Adv. Cyclic Nucleotide Protein Phosphorylation Res.* **17**, 145–151

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