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Amplification of Cyclic AMP Generation Reveals Agonistic Effects of Certain β-Adrenergic Antagonists

JEFFREY R. JASPER, MARTIN C. MICHEL, and PAUL A. INSEL

Department of Pharmacology, School of Medicine, University of California, San Diego, La Jolla, California 92093

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SUMMARY

Some β-adrenergic receptor (βAR) antagonists, in addition to blocking receptor-mediated responses, possess agonistic properties or intrinsic sympathomimetic activity (ISA). In this study we describe several techniques for amplification of cAMP levels as a measure of agonistic activity, and we apply these techniques to the study of βAR antagonists with ISA. We show that 1) a variety of βAR antagonists with ISA, including alprenolol and cyanopindolol, enhance cyclic AMP accumulation in S49 lymphoma cells if cells are also incubated with the diterpene forskolin; 2) βAR blockers with ISA stimulate cAMP accumulation in the presence of a water-soluble analog of forskolin but not in the presence of 9,11-dideoxyforskolin (which does not activate adenylyl cyclase); 3) the potentiation by forskolin is not unique to S49 cells but is also observed in BC_3H_1 smooth muscle-derived cells; 4) stimulation of cAMP accumulation by β-blockers with ISA occurs in S49 cells in three additional settings that do not involve the use of forskolin, after pretreatment with pertussis toxin to inactivate the inhibitory guanine nucleotide binding protein, after pretreatment with [D-Trp^8]-somatostatin to sensitize adenylyl cyclase, and using a radioimmunoassay to quantitate levels of cellular cAMP. We conclude that βAR antagonists with ISA can weakly stimulate intracellular cAMP accumulation, but this stimulation is not easily detected. Elevation of cAMP levels may account for the agonistic effects of these drugs or, at least provides a measure of stimulatory guanine nucleotide-binding protein activation by these compounds.

βAR antagonists are commonly used in the treatment of various disease states including angina pectoris and hypertension (1). Some data indicate that βAR antagonists with ISA may have beneficial effects compared with those without; treatment with β-blockers with ISA yields a lower incidence of hypotensive side effects (2) and does not cause withdrawal syndromes or adversely affect lipid metabolism as can antagonists without ISA (3, 4). Therefore, in many clinical studies, β-blocking agents with ISA produce as good, or better, results as those obtained with pure β-adrenergic antagonists (5, 6).

In spite of their wide use, the mechanism of action of β-blocking drugs with ISA has been unclear. In earlier studies, these compounds did not appear to stimulate adenylyl cyclase activity in several tissues (7–10). We have recently described an *in vitro* method for the study of ISA, which enabled us to demonstrate that several βAR antagonists with ISA can elevate intracellular levels of cAMP in S49 lymphoma cells (11). The technique used the diterpene forskolin to potentiate stimulation of cellular cAMP accumulation. However, forskolin can have various biological effects which are unrelated to G_s/adenylyl cyclase activation (12–14). In this paper we describe several different methods for amplifying small cAMP signals in whole cells. Applying these methods to the study of βAR blockers with ISA leads us to conclude that these drugs stimulate the G_s via the βAR and ultimately cAMP accumulation within target cells.

Experimental Procedures

Cell culture. S49 lymphoma cells were grown in DME/HS at 37° as described (15). BC_3H_1 smooth muscle-like cells or MDCK cells were grown as described previously (16, 17) and experiments were conducted after 4–6 days of confluence (≈10^6 cells/35-mm dish).

In some experiments, S49 cells (3–5 × 10^5 cells/ml) were treated with 100 ng/ml pertussis toxin (18) overnight (18 hr) under normal culture conditions. Cells were washed once in DME/HS to remove the toxin before incubation with βAR drugs. Similarly, certain experiments involved treatment of S49 cells with 10^{-6} M [D-Trp^8]-somatostatin for 18 hr, followed by a single wash and then incubation with βAR ligands.

Measurement of cAMP accumulation. Cyclic AMP accumulation in wild type S49 lymphoma cells was assessed as previously described (11). Briefly, S49 cells (2–5 × 10^5) were incubated with drugs at 37° in

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ABBREVIATIONS: βAR, β-adrenergic receptor; ISA, intrinsic sympathomimetic activity; G_s, stimulatory guanine nucleotide-binding protein; G_i, inhibitory guanine-nucleotide binding protein; ICYP, (−)-iodocyanopindolol; DME/HS, Dulbecco's modified Eagle's medium containing 10% heat-inactivated horse serum; DME/H/BSA, Dulbecco's modified Eagle's medium containing 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and 1 g/liter bovine serum albumin at pH 7.4; MDCK, Madin-Darby canine kidney; G protein, guanine nucleotide-binding protein.

1 ml of DME/H/BSA. Superoxide dismutase and catalase (10 μg/ml each) were added to prevent oxidation of drugs (19) and isobutylmethylxanthine and Ro 20-1724 (0.1 mM each) to inhibit phosphodiesterases. Some tubes contained forskolin, 9,11-dideoxy-forskolin, or the water-soluble forskolin analog. The incubation was stopped after 5 min by centrifugation at 10,000 × *g* and aspiration of the supernatant (5 min was chosen as a convenient time to elicit a cAMP response; however, results were similar at shorter and longer incubation times). The pellet was resuspended in 150 μl of stop buffer (50 mM sodium acetate containing 0.2 mM isobutylmethylxanthine, pH 4.0) and placed in a boiling water bath for 5 min. Cyclic AMP was assayed, as described, by the method of Gilman (20) with minor modifications (21).

Cyclic AMP accumulation in BC_3H_1 and MDCK cells was performed in a similar way. Before experimentation, culture medium was aspirated from the plates and the cells were incubated with DME/H/BSA for 30 min at 37°. This medium was aspirated and the cells were incubated with 1.8 ml of fresh DME/H/BSA (to reduce basal cAMP levels in the cells). Cells were then stimulated for 5 min with βAR ligands in the presence of 1 μM forskolin. Reaction was terminated by aspiration of the medium and addition of 500 μl of ice-cold stop buffer (150 mM KCl, 5 mM $KHPO_4$, 2 mM EDTA, 0.5 mM isobutylmethylxanthine, pH 6.8) to each plate, as described by Meier *et al.* (16). Plates were kept on ice and scraped with a plastic spatula. Cells were transferred to microfuge tubes, the plates washed with an additional 500 μl of stop buffer, and the microfuge tubes were placed in boiling water for 5 min. Intracellular cAMP was measured by the same method described above for S49 lymphoma cells.

In some experiments, we measured cAMP accumulation in S49 cells by radioimmunoassay. Here, S49 cells were stimulated by βAR ligands at a density of only 2×10^4 cells/ml for 5 min (in the absence of forskolin). The generated cAMP was measured with a kit from Amersham, which involved competitive binding of ^{125}I-cAMP to rabbit antibodies that are cAMP specific.

Radioligand binding. The affinity of drugs for S49 β_2AR was determined by inhibition of 30 pM [^{125}I]ICYP binding to intact cells, as previously described (22).

Chemicals. Chemicals were purchased from the following sources: (±)-pindolol and isoproterenol from Sigma (St. Louis, MO), dichloroisoproterenol from Aldrich (Milwaukee, WI), forskolin, 1,9-dideoxyforskolin, and water-soluble forskolin (7-deacetyl-7-[4-methylpiperazino] butyryloxy dihydrochloride·H_2O) from Calbiochem (La Jolla, CA), [^{125}I]ICYP and [^{3}H]cAMP from New England Nuclear (Boston, MA), [D-Trp^8]-somatostatin from Bachem (Torrance, CA), and pertussis toxin from List Biochemicals, Inc. (Campbell, CA). The following drugs were gifts of the respective companies: (+)- and (−)-pindolol, cyanopindolol, and bopindolol from Sandoz (Basel, Switzerland), xamoterol from ICI (Plankstadt, West Germany), celiprolol from Rorer (Bielefeld, West Germany), (−)-propranolol from Ayerst (New York, NY), tertatolol from Servier (Neuilly sur Seine, France), and H142/08 and alprenolol from Hässle (Mölndal, Sweden).

Data analysis. Concentration-response curves were constructed by fitting the experimental data to a sigmoid curve with computer-assisted nonlinear regression analysis; the Hill slope was fixed at unity for these calculations. K_i values for inhibition of isoproterenol-stimulated cAMP accumulation and for [^{125}I]ICYP binding were calculated from the IC_{50} values, according to the equation by Cheng and Prusoff (23). For these conversions, we used an EC_{50} of isoproterenol of 30 nM and a K_d for [^{125}I]ICYP of 12 pM. Significance of differences was assessed by two-tailed *t* tests, and a *p* value of less than 0.05 was considered to be significant.

Results

Affinity of β-blockers with ISA correlates with the EC_{50} values for stimulatory response. In initial experiments that we reported recently (11), we found that several βAR antagonists with ISA can enhance cAMP accumulation in wild type S49 lymphoma cells that are simultaneously incubated with 1 μM forskolin. It has been proposed that the agonistic effects of β-blockers with ISA are caused by an "atypical" mechanism (24, 25). If this is the case, one might expect that the agonistic potency of these drugs might differ from their antagonistic potency. Therefore, we determined the affinity (by inhibition of [^{125}I]ICYP binding), the antagonistic potency (by inhibition of isoproterenol-stimulated cAMP accumulation in the absence of forskolin), and the agonistic potency (cAMP accumulation in the presence of forskolin) for a number of structurally different βAR antagonists with ISA in S49 cells. None of these drugs significantly stimulated cAMP accumulation in the absence of forskolin (data not shown), but all inhibited isoproterenol-stimulated cAMP accumulation (Table 1). In the presence of forskolin, they had variable degrees of agonistic effects. The agonistic potencies correlated with the antagonistic potencies (Fig. 1A) as well as the βAR affinities of these compounds (Fig. 1B). The antagonistic potencies also correlated well with the affinities determined from the binding studies (Fig. 1C). Interestingly, the agonistic and antagonistic effects of pindolol showed the same stereoselectivity (Table 1). Also, the "antagonist" ligands cyanopindolol and alprenolol, which are often used in βAR binding studies, weakly stimulated cAMP accumulation in S49 cells.

Responsiveness of cells to partial agonists requires a functional G_s protein. The G_s protein transduces the stimulatory signal between the βAR and adenylyl cyclase (26). Thus, if stimulation of cAMP accumulation by β-blocking drugs with ISA occurs via the expected rather than an atypical mechanism (24, 25), a G_s protein should be required. We found that cyc$^-$ and UNC S49 cells do not exhibit forskolin-potentiated cAMP accumulation in response to β-blockers with ISA (Ref. 11 and Fig. 2). The cyc$^-$ mutant completely lacks the $G_s\alpha$ protein (27), whereas UNC expresses a mutated G_s protein incapable of functionally interacting with the βAR on these cells (28). These data demonstrate the necessity of G_s for responsiveness to βAR partial agonists.

Potentiation of cAMP accumulation by forskolin analogs. Forskolin can have effects not related to its action on the G protein/adenylyl cyclase system (12–14). Therefore, we used forskolin analogs to test whether other actions of forskolin might be responsible for the potentiation of cAMP accumulation. Because forskolin is quite hydrophobic and requires ethanol or dimethylsulfoxide for dissolution, the possibility exists that nonspecific chaotropic effects on the plasma membrane might be responsible for the enhanced cAMP accumulation in the presence of forskolin. We found that a water-soluble derivative of forskolin could also potentiate cAMP accumulation by isoproterenol in S49 lymphoma cells and could reveal stimulation by dichloroisoproterenol, pindolol, and celiprolol (Fig. 3A). In these studies, propranolol did not stimulate cAMP accumulation in excess of levels achieved by the forskolin analogue alone. Additionally, we tested another derivative of forskolin, 1,9-dideoxyforskolin, which is as hydrophobic as the parent compound yet is inactive in stimulating adenylyl cyclase (13). 1,9-Dideoxyforskolin did not potentiate isoproterenol-stimulated cAMP accumulation nor did it elicit a response by the β-blockers with ISA (Fig. 3B).

Enhanced responsiveness in BC_3H_1 cells by forskolin. In order to test whether the effects of forskolin observed in S49 lymphoma cells are unique to this cell type, we examined BC_3H_1

TABLE 1

Efficacy, agonistic and antagonistic potency, and affinity of various β-blockers with ISA

The agonistic efficacy is shown in the first column and is expressed as maximal stimulation of cAMP accumulation (pmol of cAMP/10^7 cells/5 min) in S49 lymphoma cells elicited by the respective βAR ligands in the presence of 10^{-6} M forskolin. The agonistic potency is shown in the second column and is expressed as the −log of the EC_{50} (pEC_{50}). The antagonistic potency is shown in the third column and is expressed as the −log of the K_i (pK_i) for inhibition of 10^{-7} M isoproterenol-stimulated cAMP accumulation. The last column shows the affinities for S49 cell βAR as determined from binding studies and expressed as the −log of the K_i (pK_i). All values are the average of three to five independent experiments and are shown as mean ± standard error. No significant stimulation of cAMP generation was found for tertatolol or propranolol. The reason for the different efficacy and potency of (±)-pindolol, compared with its isomers, is not clear; it might be related to differences in purity because the racemic drug was obtained from a different source than the isomers. Data for propranolol, dichloroisoproterenol, and celiprolol are pooled from previously published (11) and some new experiments. Data for 8-hydroxycarteolol are from Ref. 45 and are shown for comparison.

	Max cAMP	pEC_{50} (cAMP)	pK_i (cAMP)	pK_i (binding)
	pmol/10^7 cells/5 min			
Isoproterenol	1708 ± 360	8.00 ± 0.35		ND[a]
8-Hydroxycarteolol	297 ± 52	8.00 ± 0.26	7.84 ± 0.30	ND
Dichloroisoproterenol	233 ± 52	7.13 ± 0.20	7.27 ± 0.15	7.35 ± 0.03
H142/08	207 ± 47	6.07 ± 0.23	5.58 ± 0.11	5.69 ± 0.02
(−)-Pindolol	151 ± 17	8.52 ± 0.52	8.62 ± 0.16	9.23 ± 0.07
(+)-Pindolol	143 ± 19	7.53 ± 0.04	7.26 ± 0.25	7.23 ± 0.05
Celiprolol	127 ± 15	6.74 ± 0.06	5.72 ± 0.37	5.83 ± 0.05
(±)-Pindolol	83 ± 27	9.49 ± 0.05	9.12 ± 0.14	9.26 ± 0.13
Xamoterol	69 ± 14	6.57 ± 0.17	5.70 ± 0.03	5.94 ± 0.04
Bopindolol	65 ± 22	8.16 ± 0.59	9.19 ± 0.40	8.81 ± 0.06
Cyanopindolol	49 ± 13	8.50 ± 0.25	9.40 ± 0.10	ND
Alprenolol	48 ± 10	9.21 ± 0.42	9.09 ± 0.75	9.00 ± 0.07
Tertatolol	0		8.85 ± 0.13	ND
Propranolol	0		9.48 ± 0.17	9.62 ± 0.08

[a] ND, not determined.

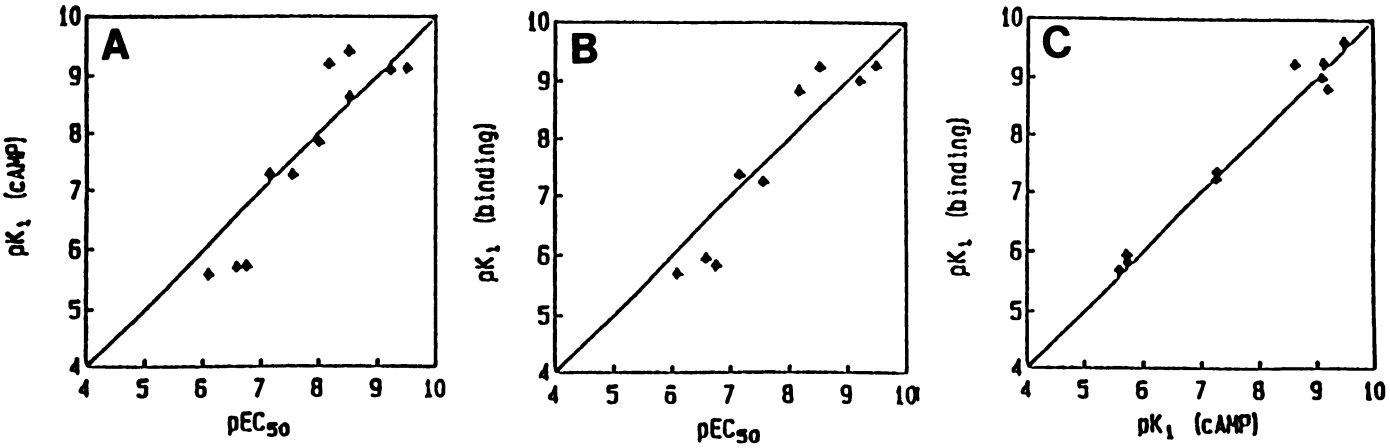

Fig. 1. Correlation of agonistic and antagonistic potency and affinity of β-blockers with ISA. Data are taken from Table 1 and expressed as −log of the EC_{50} values for the stimulation of cAMP in the presence of 10^{-6} M forskolin and of the K_i values for either inhibition of (−)-isoproterenol-stimulated cAMP (cAMP) or inhibition of [^{125}I]ICYP binding. A, pEC_{50} versus pK_i for inhibition of agonist-stimulated cAMP; B, pEC_{50} versus pK_i for inhibition of [^{125}I]ICYP binding; C, pK_i versus values for cAMP versus pK_i values for [^{125}I]ICYP binding. In each panel, the *line of identity* is plotted for convenient comparison.

cells, a cell line that is derived from murine smooth muscle and that also expresses β_2AR (29, 30). In these cells, β-blockers with ISA (celiprolol, dichloroisoproterenol, and pindolol) did not significantly enhance cAMP accumulation by themselves (data not shown). Forskolin (1 μM) potentiated cyclic AMP accumulation in response to isoproterenol and revealed stimulation by the β-blockers with ISA (Fig. 4); propranolol or tertatolol did not stimulate cAMP accumulation in the absence or presence of forskolin (data not shown). The potentiation of cAMP accumulation by forskolin, however, does not occur in all cell types. Forskolin did not potentiate isoproterenol-stimulated cAMP accumulation in human lymphocytes (31) or in MDCK cells (data not shown). Accordingly, β-blockers with ISA did not significantly stimulate cAMP generation in the absence or the presence of forskolin in these two cell types (Ref. 9 and data not shown).

Amplification of βAR ligand-stimulated cAMP levels by pertussis toxin treatment. As an alternative to the use of forskolin, we tested other means to amplify cAMP accumulation. We investigated the effects of a reduction in the activity of the inhibitory G protein, G_i, by pertussis toxin on cAMP accumulation in S49 cells. Overnight treatment with 100 ng/ml pertussis toxin enhanced isoproterenol-stimulated cAMP accumulation (Fig. 5, *inset*). In pertussis toxin-treated cells, dichloroisoproterenol, pindolol, and celiprolol significantly stimulated cAMP accumulation in the absence of forskolin; propranolol, which lacks ISA, had no stimulatory effects (Fig. 5). Additionally, 1 μM propranolol abolished the enhanced cAMP accumulation produced by β-blockers with ISA in pertussis toxin-treated cells (Fig. 5).

[D-Trp^8]-Somatostatin sensitization of adenylyl cyclase in S49 cells. Recent data have demonstrated that a 24-

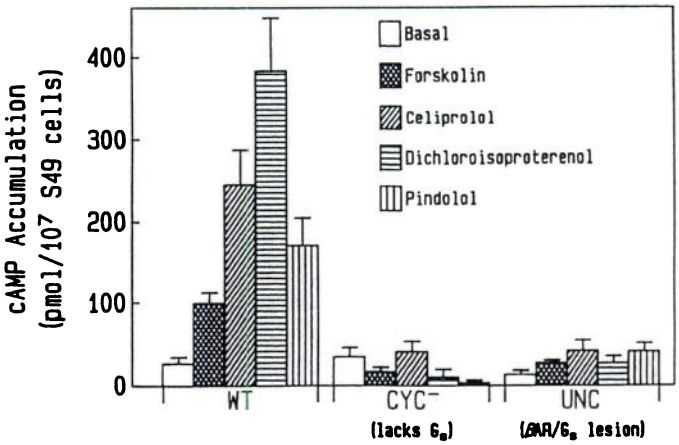

Fig. 2. Forskolin-potentiated cAMP accumulation by βAR antagonists with ISA depends on a functional G_s. Wild type (*WT*), cyc⁻, and UNC S49 lymphoma cells were incubated with forskolin and the βAR ligands celiprolol, dichloroisoproterenol, pindolol, propranolol, or isoproterenol, as described in Experimental Procedures, and cAMP accumulation was measured after 5 min. Shown is the average of three independent experiments.

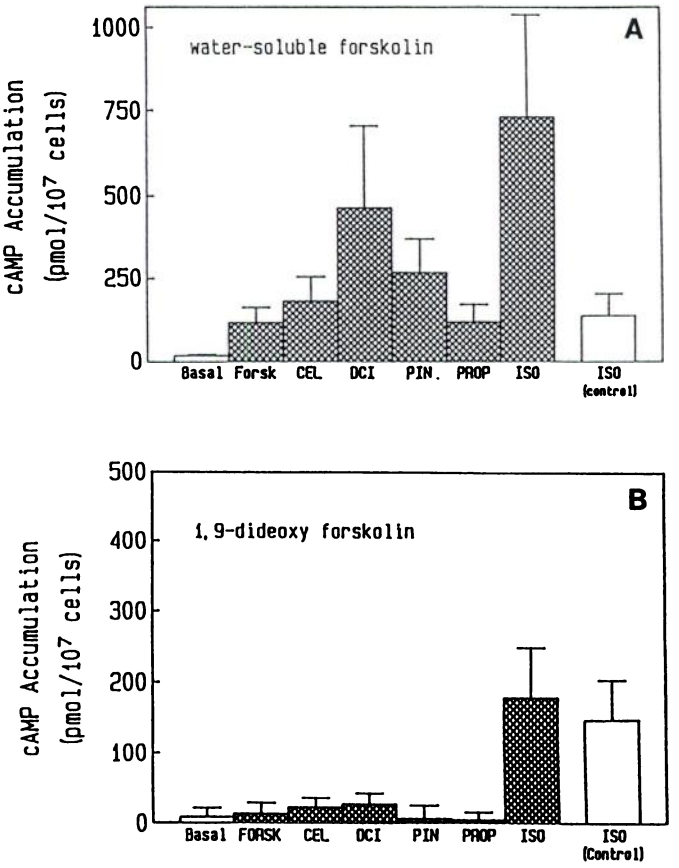

Fig. 3. Maximal stimulation of cAMP accumulation in S49 lymphoma cells by βAR antagonists with ISA: effects of water-soluble and inactive forskolin (*Forsk*) derivatives. Cells were incubated with water-soluble forskolin (10^{-5} M) (A) or 1,9-dideoxyforskolin (10^{-5} M) (B) and the βAR ligands celiprolol (10^{-4} M) (*CEL*), dichloroisoproterenol (10^{-5} M) (*DCI*), pindolol (10^{-6} M) (*PIN*), isoproterenol (10^{-6} M) (*ISO*), or propranolol (10^{-6} M) (*PROP*). All values are the mean ± standard error of three independent experiments.

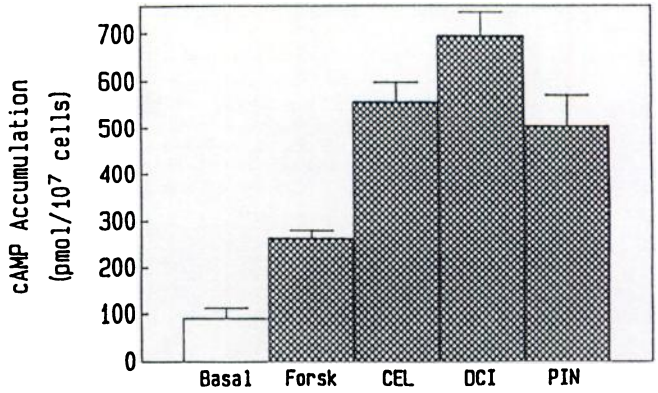

Fig. 4. Cyclic AMP accumulation in BC_3H_1 smooth muscle cells. BC_3H_1 cells were incubated with βAR ligands, as described in Fig. 2, in the presence of 10^{-6} M forskolin. Shown is the mean ± standard error of three independent experiments. Abbreviations are as in Fig. 3.

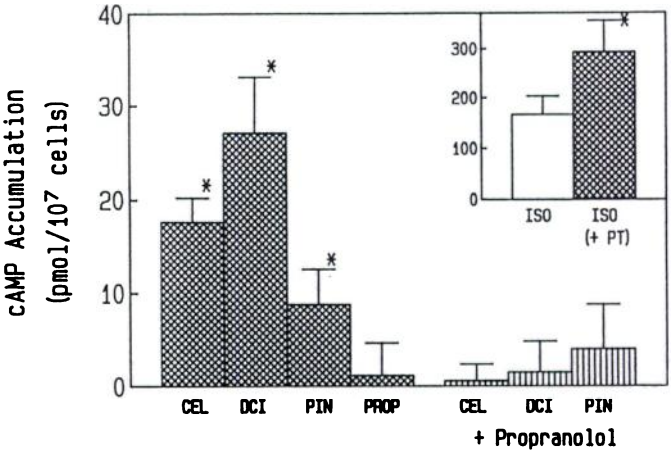

Fig. 5. Stimulation of cAMP accumulation in S49 lymphoma cells following pertussis toxin treatment. S49 lymphoma cells were treated with pertussis toxin (100 ng/ml) for 16–18 hr under normal growth conditions. Following a single wash, cells were incubated with βAR ligands (as in Fig. 2) in the absence of forskolin and in the absence (■) or presence (■) of 1 μM (−)-propranolol. Shown is the mean of nine independent experiments, with basal cAMP levels substracted from each value. *Inset*, stimulation of cAMP accumulation by (−)-isoproterenol in control and pertussis toxin (*PT*)-treated cells. *$p < 0.05$ versus basal cAMP accumulation. Abbreviations are as in Fig. 3.

hr treatment of S49 cells with the somatostatin receptor agonist SMS 201-995 enhances βAR-stimulated adenylyl cyclase activation (32). We have applied this paradigm to the study of βAR antagonists with ISA. Similar to the findings of Thomas and Hoffman (32), we found that a 16–18-hr incubation of wild type S49 lymphoma cells with the stable somatostatin analogue [D-Trp⁸]-somatostatin (1 μM) slightly enhanced 1 μM isoproterenol-stimulated cAMP generation (not shown). In cells treated with the somatostatin analog, celiprolol, dichloroisoproterenol, and pindolol also significantly stimulated cAMP accumulation, whereas propranolol had no effect (Fig. 6).

Measurement of cAMP accumulation by radioimmunoassay. We used a sensitive radioimmunoassay to test whether stimulation of cAMP accumulation by β-blockers with ISA could also be demonstrated without any pretreatment or coincubation of cells. This radioimmunoassay yields more accurate and precise determinations of cAMP than does the competitive protein binding assay at the basal concentrations of cAMP typically observed in S49 cells (data not shown). With

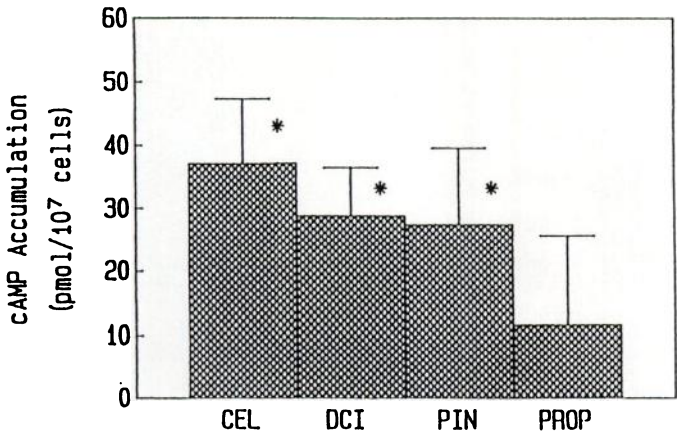

Fig. 6. Stimulation of cAMP accumulation in S49 lymphoma cells following treatment with [D-Trp^8]-somatostatin. After 16–18 hr of treatment under normal growth conditions, the cells were washed once. Then βAR ligands were added as described in Fig. 2, and cAMP accumulation was determined. Shown is the mean of five experiments ± standard error, with basal cAMP levels subtracted (basal = 18 ± 7 pmol of cAMP/10^7 cells). *$p < 0.05$ versus basal cAMP accumulation. Abbreviations are as in Fig. 3.

this technique, we found that celiprolol and dichloroisoproterenol stimulated cAMP accumulation in S49 lymphoma cells very weakly over basal levels (celiprolol, 28 ± 9.6 pmol/10^7 cells, four experiments, $p = 0.06$; dichloroisoproterenol, 70 ± 19 pmol/10^7 cells, four experiments, $p = 0.03$) in the absence of forskolin; the antagonist propranolol did not stimulate cAMP accumulation over basal (7.6 ± 8 pmol/10^7 cells, two experiments), whereas the full agonist isoproterenol elicited a large cAMP response (609 ± 129 pmol/10^7 cells, two experiments). The increases in cAMP produced by the β-blockers with ISA are consistent with the other findings in S49 cells produced by treatments that amplify βAR receptor-stimulated cAMP accumulation.

Discussion

Various investigators have been unable to demonstrate stimulation of cAMP formation by β-blockers with ISA (7–10). Because cAMP is believed to be the principal effector of βAR-mediated effects, it was postulated from these data that ISA might be mediated by a different atypical pathway (8, 24, 25, 33). In contrast, the current results provide persuasive evidence that some β-blockers with ISA can stimulate cAMP generation. This conclusion is supported by five approaches: 1) testing several different β-blockers and using different analogs of forskolin, 2) using two different cell lines, wild type S49 lymphoma cells and BC_3H_1 muscle cells, 3) inactivating G_i with pertussis toxin, 4) sensitizing cAMP accumulation with somatatostatin, and 5) using a radioimmunoassay to examine low levels of cAMP accumulation.

Although stimulation of cAMP generation by β-blockers with ISA in the presence of forskolin was demonstrated in both S49 lymphoma and BC_3H_1 cells, we did not observe such stimulation in the presence of forskolin in human peripheral mononuclear leukocytes or in MDCK cells. In both of these types of cells, forskolin also fails to significantly potentiate adenylyl cyclase activation by isoproterenol. Because human peripheral mononuclear leukocytes and S49 cells appear to contain approximately the same number of βAR (~1000–1500/cell), the difference in responsivity to forskolin and β-antagonists cannot be accounted for by a difference in receptor number. We speculate that there are different amounts of G_s protein or amplification between receptors and G_s, which would facilitate synergy by forskolin between β-receptor agonists, G_s, and adenylyl cyclase in the different cell types (12). The exact mechanism for such synergy is not known; however, it is probably related to the capability of forskolin to stimulate adenylyl cyclase, perhaps by enhancement in activity of the catalyst of the enzyme or interaction with the G_s protein, and not to nonspecific membrane perturbations by forskolin.

It is believed that the βγ subunits of G_i mediate, at least partially, inhibition of adenylyl cyclase activity (26). Therefore, tissues that express large amounts of G_i may exhibit a tonic inhibition of the catalytic unit of adenylyl cyclase. Inactivation of G_i in such cell types by treatment with pertussis toxin will release the enzyme from this tonic inhibition and, thus, potentiate agonist-stimulated cAMP generation (34–36). We used this paradigm as one approach to demonstrate that β-blockers with ISA stimulate cAMP accumulation in pertussis toxin-treated S49 cells even in the absence of forskolin.

Another model of enhanced adenylyl cyclase responsiveness involves desensitization of the G_i pathway by treatment with agonists at receptors coupling to inhibition of adenylyl cyclase, e.g., the somatostatin receptor (37). We have extended these observations by using the somatostatin receptor agonist [D-Trp^8]-somatostatin. Overnight treatment of S49 cells with this stable somatostatin analog sensitized adenylyl cyclase to the stimulatory effects of isoproterenol and revealed stimulation of cAMP generation by β-blockers with ISA.

These data suggest that release of tonic inhibition of adenylyl cyclase enables one to detect enzyme stimulation by βAR antagonists with ISA. An alternative explanation of our data would be that β-blockers with ISA simultaneously stimulate adenylyl cyclase activity via G_s and inhibit it via G_i. Although the possibility of βAR/G_i interactions has been suggested by some data (38, 39), we believe that the former explanation is more likely, because we fail to find substantial evidence for βAR interactions in wild type S49 cells that possess their full complement of G_s and G_i (40). Our explanation is also supported by the detection of weak stimulation of cAMP generation by β-blockers with ISA with a radioimmunoassay.

It has been proposed that some agonistic effects of β-blockers with ISA might not be mediated by the βAR but instead by other cell surface molecules (24, 25). Such proposals were supported by observations suggesting that only a portion of the sympathomimetic activity of these β-blockers was blocked by propranolol (41) and that the concentration-response curve for the positive chronotropic effect of pindolol in guinea pig myocardium was biphasic (24). When we compared the agonistic potencies of various structurally distinct βAR antagonists possessing different degrees of ISA with their affinities and also with their antagonistic potencies at the S49 cell βAR, we found that agonistic and antagonistic potencies as well as receptor affinities are very similar for each compound tested. Thus, our data do not support the concept of βAR-independent effects of β-blockers with ISA.

Although our data show that βAR antagonists with ISA can stimulate cAMP generation, they do not unequivocally prove that this is the mechanism of their agonistic effects at the βAR. Some data suggest that βAR stimulation can generate second messengers distinct from adenylyl cyclase activation. These include inhibition of Mg^{2+} influx in S49 lymphoma cells (42), activation of Ca^{2+} channels in the heart (43), and activation of chloride ion secretion in canine tracheal cells (44). Therefore,

it is possible that some of the agonistic effects of β-blockers with ISA are not related to activation of adenylyl cyclase but instead to stimulation of other second messengers of the βAR. Moreover, inhibition of Mg^{2+} influx and activation of Ca^{2+} channels also appear to be mediated via G_s and stimulation of cAMP accumulation in S49 cells by β-blockers with ISA depends on the presence of intact G_s (Ref. 11 and Fig. 2). Viewed in this way, the detection of cAMP accumulation by β-blockers with ISA appears to provide a readily detectable measure of G_s activation. Thus, we believe that these compounds are capable of promoting responses in target cells because of their ability to promote interaction of βAR with the G_s protein. Whether the resultant increase in cyclic AMP accumulation is the exclusive mechanism for physiological responses will require additional study.

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Send reprint requests to: Paul A. Insel, Department of Pharmacology, M-036, School of Medicine, University of California, San Diego, La Jolla, CA 92093.