Proc. Natl. Acad. Sci. USA
Vol. 75, No. 11, pp. 5362–5366, November 1978
Biochemistry

Adenosine analogs inhibit adipocyte adenylate cyclase by a GTP-dependent process: Basis for actions of adenosine and methylxanthines on cyclic AMP production and lipolysis

(membrane receptors/purine-modified/ribose-modified)

CONSTANTINE LONDOS, DERMOT M. F. COOPER, WERNER SCHLEGEL, AND MARTIN RODBELL

National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bldg. 6, Room B1-22, Bethesda, Maryland 20014

Communicated by DeWitt Stetten, Jr., August 17, 1978

ABSTRACT **Adenylate cyclase in purified membranes from rat adipocytes is inhibited by low concentrations of purine-modified adenosine analogs, particularly those modified in the N^6 position. Such inhibition is antagonized competitively by methylxanthines, but not by other cyclic nucleotide phosphodiesterase inhibitors, and it is dependent on "inhibitory" concentrations of GTP in the assay medium. Ribose-modified adenosine analogs inhibit adenylate cyclase through a process that is neither dependent upon the GTP concentration nor antagonized by methylxanthines. These results explain the potent effects of adenosine and methylxanthines on fat cell metabolism and demonstrate the importance of GTP in mediating inhibition by agents that act at cell surface receptors.**

Two distinct sites through which adenosine alters adenylate cyclase activity in plasma membrane preparations have been identified with the use of adenosine analogs (1). One site mediates inhibition in all cases thus far described and accepts, as agonists, adenosine analogs modified in the ribose moiety, such as 2',5'-dideoxyadenosine and 9-β-D-arabinofuranosyladenine. This site does not react with several purine-modified analogs and, based on the requirement for integrity of the purine moiety, has been designated the P site. The other site, termed R, does not react with most ribose-modified analogs but accepts purine-modified compounds such as N^6-phenylisopropyladenosine, N^6-methyladenosine, and 2-methyladenosine. In all cases described thus far, the R site mediates activation of adenylate cyclase. Methylxanthines, such as theophylline, antagonize the activating effects of adenosine at the R site but have no effect on the P site. These generalizations are based on a comparison of our direct examination of several adenylate cyclases (1) with numerous published reports on the effects of adenosine and adenosine analogs on cyclic AMP accumulation in intact cells and on adenylate cyclase activity (2–17, plus others cited in ref. 1).

The rat adipocyte presents a paradox with regard to the effects of adenosine analogs in that both P and R site-reactive compounds depress cyclic AMP accumulation in the intact cell (18–20). P site effectors, such as 2',5'-dideoxyadenosine, depress adenylate cyclase activity in isolated membrane preparations (18, 19, 21). However, R site effectors, such as N^6-methyladenosine and N^6-phenylisopropyladenosine, which are highly potent inhibitors of cyclic AMP formation and lipolysis in the cell, have not been shown to modify adenylate cyclase activity in isolated membranes (18–21). Yet, as with the systems discussed above, methylxanthines antagonize the actions of low concentrations of adenosine and of R site-reactive analogs on the fat cell (19–23), suggesting the presence of an R site. Therefore, we have reexamined the fat cell adenylate cyclase to attempt to find conditions that would permit inhibition by R site-reactive analogs.

This study shows that GTP plays an essential role in the expression of R site-mediated inhibition of adenylate cyclase activity in adipocyte plasma membranes. With the fat cell enzyme, GTP exerts both stimulatory and inhibitory effects (23–26) that appear to be expressed through different processes (26). Our data show that inhibition by R site analogs is linked to the GTP inhibitory process, occurs with those analogs that potently inhibit cyclic AMP formation and lipolysis in fat cells, and is antagonized by methylxanthines. These findings can explain the potent inhibitory effects of adenosine on fat cell metabolism and the stimulatory effects of methylxanthines on cyclic AMP formation and lipolysis.

MATERIALS AND METHODS

The sources of materials used in the adenylate cyclase assay have been reported (27). Theophylline, caffeine, papaverine·HCl, L-isoproterenol-D-bitartrate, 2-chloroadenosine, 9-β-D-ribofuranosylpurine, and calf intestinal adenosine deaminase (230 units/ml) were purchased from Sigma. N^6-Methyladenosine, 2-methyladenosine, 2-aminoadenosine, and 9-β-D-arabinofuranosyladenine were gifts from the International Chemical and Nuclear Corp. N^6-Cyclohexyladenosine and N^6-propyladenosine were gifts from Boehringer Mannheim. 2'-Deoxyadenosine and 5'-deoxyadenosine were obtained from P-L Biochemicals. 2',5'-Dideoxyadenosine and 9-β-D-xylofuranosyladenine were provided by the Drug Research and Development Branch of the National Cancer Institute. N^6-Phenylisopropyladenosine was from J. N. Fain (Brown University). RO-20-1724, which is *d*-4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone, was from Hoffmann–La Roche. 1-Methyl-3-isobutylxanthine was bought from Aldrich. The ATP used in these studies was either purified by M. C. Lin according to the method of Kimura *et al.* (28) or the Sigma product (A-2383) prepared by phosphorylation of adenosine. Both preparations were sufficiently free of GTP contamination to permit detectable effects of 2 nM GTP added to the adenylate cyclase assay medium.

Preparation of Fat Cell Membranes. Plasma membranes were isolated by a simplification of the method of Avruch and Wallach (29), suspended in 1 mM EDTA/10 mM Tris·HCl, pH 7.5, and stored in liquid N_2 as described by Harwood *et al.* (23).

Adenylate Cyclase Assay. Adenylate cyclase activity was assayed by the method of Salomon *et al.* (27) in a medium containing 0.1 mM ATP, 1 μCi of [α-^{32}P]ATP, 4 mM $MgCl_2$, 0.1 mM cyclic AMP, 2 mM creatine phosphate, creatine phosphokinase at 25 units/ml, 30 mM Tris·HCl (pH 7.5), and 0.1% crystalline bovine serum albumin. Reactions were initiated by the addition of approximately 1 μg of membrane protein to give

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

a total volume of 0.1 ml. Incubations were carried out for 30 min at 24°C, conditions that optimize GTP sensitivity of the fat cell cyclase (unpublished data). Experiments were performed in duplicate or triplicate with three batches of fat cell plasma membranes.

RESULTS

The typical biphasic effect of GTP on hormone-activated fat cell adenylate cyclase is shown in Fig. 1. In the presence of isoproterenol, peak activity was obtained with approximately 100 nM GTP, and higher concentrations decreased both basal and hormone-stimulated activities. Also shown are the effects of two adenosine analogs, N^6-methyladenosine and N^6-phenylisopropyladenosine, known to be potent inhibitors of lipolysis and cyclic AMP formation in rat adipocytes (19–21, 30). The nucleosides had no effect on the ascending portion but were clearly inhibitory on the descending leg of the GTP curve. Thus, inhibitory levels (with respect to peak activity) of GTP were required to obtain inhibition of adenylate cyclase with adenosine analogs that generally activate other adenylate cyclases and have been designated as R site effectors (1). Similar results were obtained with corticotropin as the stimulating hormone (data not shown). Inhibitory concentrations of GTP were included in all further experiments with R site analogs.

The experiments shown in Fig. 1 were conducted in the presence of adenosine deaminase in order to clear the medium of inhibitory levels of adenosine produced by degradation of ATP and cyclic AMP. Table 1 shows that both theophylline and adenosine deaminase increased adenylate cyclase activity, the former by antagonizing the adenosine inhibitory effect (see below) and the latter by converting adenosine to inosine which was not inhibitory. Therefore, adenosine deaminase was included in most experiments reported herein.

Table 1. Effects of adenosine deaminase and theophylline on isoproterenol-stimulated fat cell adenylate cyclase activity

Adenosine deaminase, unit/ml	Adenylate cyclase activity, pmol cyclic AMP/mg protein/min	
	No theophylline	250 μM theophylline
None	133 ± 4	164 ± 5
0.4	189 ± 5	183 ± 4
1.0	188 ± 4	193 ± 12

The assay medium contained 2.2 μg of membrane protein per ml, 1 μM isoproterenol, and 0.5 μM GTP. The adenosine deaminase was diluted directly from a stock suspension of 2550 units/ml in 3.2 M $(NH_4)_2SO_4$. Because the diluted $(NH_4)_2SO_4$ had no effect on activity, dialysis was not necessary. Values shown are the means and ranges of duplicate determinations.

Methylxanthines generally antagonize the activating effects of adenosine and R site-reactive analogs but have no effect on the inhibition by adenosine and P site-reactive analogs. The effects of theophylline on inhibition by N^6-methyladenosine and 2′,5′-dideoxyadenosine, two analogs that clearly discriminate between P and R sites (1), are shown in Fig. 2. The inhibition by N^6-methyladenosine was antagonized competitively by theophylline; the K_i for methylxanthine calculated from a

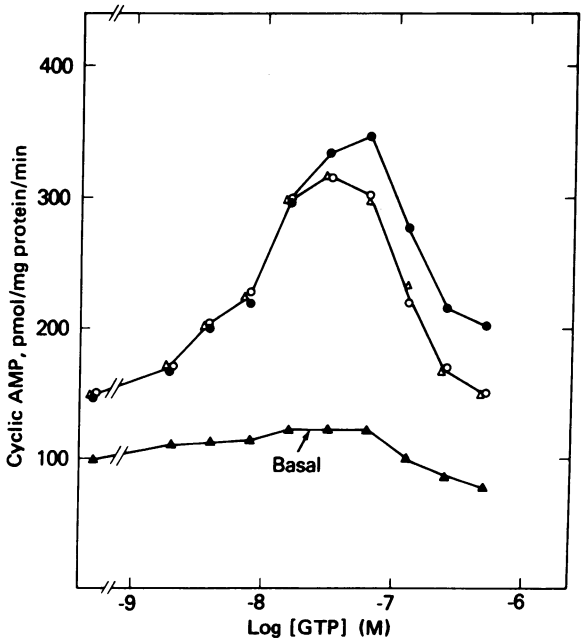

FIG. 1. Effects of N^6-phenylisopropyladenosine and N^6-methyladenosine on isoproterenol-stimulated fat cell adenylate cyclase activity as a function of GTP concentration. The assay medium contained 11 μg of membrane protein and 0.1 unit of adenosine deaminase per ml. ▲, Basal activity without hormone; ●, 1 μM isoproterenol alone; ○, isoproterenol plus 50 μM N^6-phenylisopropyladenosine; △, isoproterenol plus 50 μM N^6-methyladenosine. Basal activity was slightly inhibited by the two N^6-substituted analogs in this experiment (not shown). Note that a fairly constant increment of hormone-stimulated activity was inhibited by the analogs. In other experiments it was found that the percentage inhibition of the hormone response increased as the hormone concentration was lowered and reached a maximum of approximately 70% when the hormone concentration was sufficient to double the basal activity. However, there was always a finite increment of activity elicited by isoproterenol that could not be inhibited by R site-reactive adenosine analogs.

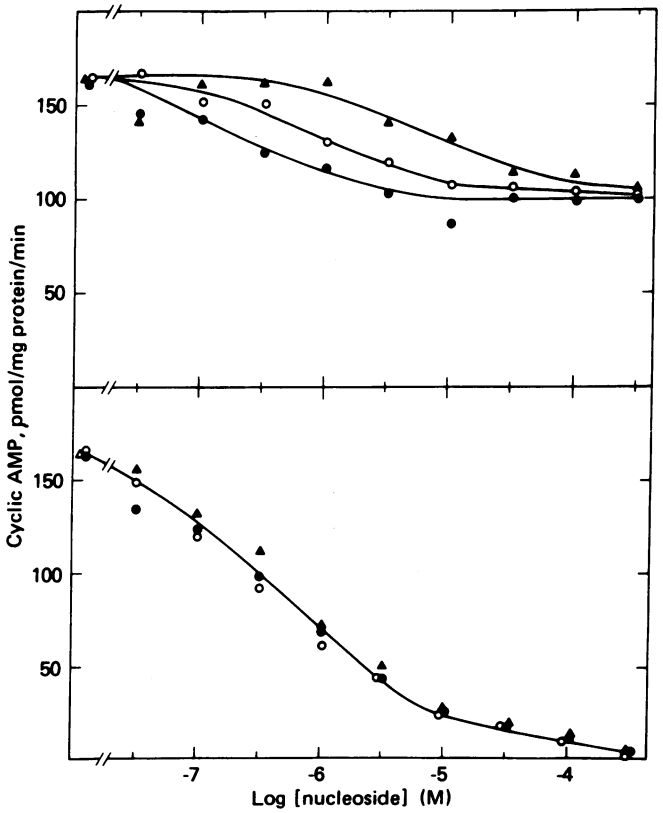

FIG. 2. Effects of theophylline on the inhibition of fat cell adenylate cyclase activity by N^6-methyladenosine (*Upper*) and 2′,5′-dideoxyadenosine (*Lower*). The assay medium contained 11 μg of membrane protein per ml, 0.1 μM isoproterenol, 10 μM GTP, and 0.1 unit of adenosine deaminase per ml. Basal activity was decreased from 60 to 45 pmol of cyclic AMP per mg of protein per min by 10 μM N^6-methyladenosine (not shown). Theophylline concentrations: ●, 0; ○, 25 μM; ▲, 250 μM.

Schild plot (31) of these data was 7 μM. On the other hand, theophylline had no effect on the action of 2′,5′-dideoxyadenosine. Thus, as with other adenylate cyclase systems, theophylline antagonized the actions of R, but not P, site effectors. Additionally, inhibition by 2′,5′-dideoxyadenosine was totally independent of the GTP concentration (data not shown), which is in contrast to the GTP-dependence for inhibition by the N^6-substituted analogs. Note also that N^6-methyladenosine reached an intermediate plateau of inhibition, whereas the 2′,5′-dideoxyadenosine completely abolished activity. As discussed below, the extent of inhibition by various analogs seems also to discriminate between P and R site effectors.

Several adenosine analogs mimicked N^6-methyladenosine in that they inhibited only when combined with inhibitory concentrations of GTP (as in Fig. 1), their effects were antagonized by theophylline, and the inhibition was not total but reached an intermediate plateau. Those compounds (N^6-phenylisopropyladenosine, purine-D-riboside, and 2-aminoadenosine), as demonstrated previously (1), are R site effectors that do not act at P sites. Analogs that mimicked 2′,5′-dideoxyadenosine—in that inhibition was independent of the GTP concentration, was not antagonized by theophylline, and did not reach a plateau—were 9-β-D-arabinofuranosyladenine, 9-β-D-xylofuranosyladenine, and 2′-deoxyadenosine. These compounds inhibit at P sites but have little or no R site activity (1). Thus, the GTP dependency, theophylline antagonism, and extent of inhibition discriminate clearly between inhibition by P and by R effectors.

Some compounds, such as adenosine and 2-chloroadenosine, react with R sites at low concentrations and with P sites at higher concentrations (1). Inhibition by 2-chloroadenosine, and the effects of theophylline thereon, are shown in Fig. 3. Theophylline antagonized the inhibition at low concentrations but not at high concentrations, which demonstrates the dual actions of 2-chloroadenosine and provides another parallel between the P and R sites of fat cells and other cell types.

We have assessed the inhibitory potencies on fat cell cyclase of a series of adenosine analogs known to be potent inhibitors of cyclic AMP formation and lipolysis in intact adipocytes (19–21). The K_i values of these compounds are: N^6-propyladenosine, 5 nM; 2-chloroadenosine, 10 nM; N^6-cyclohexyladenosine, 10 nM; N^6-phenylisopropyladenosine, 20 nM; and N^6-methyladenosine, 200 nM. The remarkable sensitivity to these analogs is similar to that observed in the intact cell (19–21).

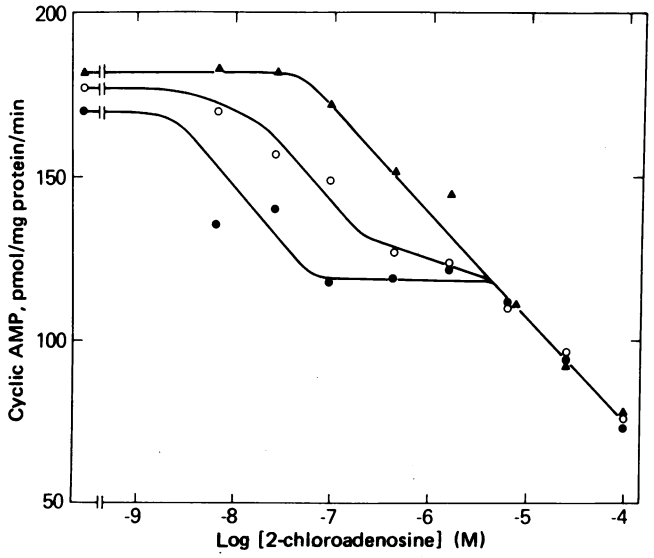

FIG. 3. Effects of theophylline on the inhibition of fat cell adenylate cyclase by 2-chloroadenosine. The assay medium contained 2.2 μg of membrane protein per ml, 2 μM GTP, 1 μM isoproterenol, and 0.1 unit of adenosine deaminase per ml. Theophylline concentrations: ●, 0; ○, 25 μM; ▲, 250 μM.

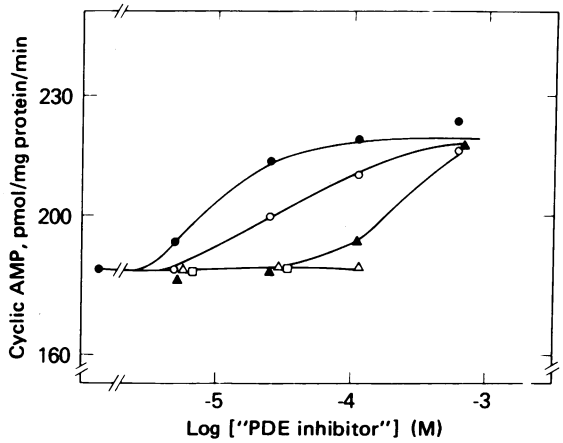

FIG. 4. Effects of several cyclic nucleotide phosphodiesterase inhibitors on the inhibition of fat cell adenylate cyclase by N^6-methyladenosine. The assay medium contained 11 μg of membrane protein per ml, 0.5 μM isoproterenol, 10 μM GTP, 1 μM N^6-methyladenosine, 0.01 mM ATP, and no cyclic AMP or adenosine deaminase. Test compounds were 1-methyl-3-isobutylxanthine (●), theophylline (○), caffeine (▲), RO-20-1724 (△), and papaverine·HCl (□).

The actions of theophylline described above were not due to inhibition of cyclic nucleotide phosphodiesterase activity. First, no hydrolysis of cyclic [^{3}H]AMP was observed over the concentration range of 12 nM to 300 μM under typical adenylate cyclase assay conditions (usually less than 10 μg of membrane protein per ml). Also, as a precaution, 100 μM cyclic AMP was included in the assay medium. Finally, of five phosphodiesterase inhibitors tested (Fig. 4), only the methylxanthines increased activity when the cyclase was inhibited with N^6-methyladenosine.

DISCUSSION

This report shows that the fat cell adenylate cyclase system contains two sites for adenosine action, both of which mediate inhibition of activity. The R site accepts purine-modified analogs, as agonists, is blocked by methylxanthines, and requires "inhibitory" concentrations of GTP for expression of activity. The second adenosine site, termed P site, also inhibits cyclase activity, accepts only ribose-modified analogs, and is neither dependent on the concentration of GTP nor blocked by methylxanthines. The apparent unique feature of the adipocyte cyclase system is that R site effectors mediate inhibition whereas in other tissues they are cyclase activators (1).

Our findings can explain the actions of adenosine and its analogs on intact fat cells reported by Fain and colleagues (18–20), Schwabe and coworkers (22, 32, 33), and Trost and Stock (21). Summarized briefly, these investigators found that fat cells extrude adenosine which, in turn, strongly inhibits cyclic AMP production and lipolysis. These effects are reversed by methylxanthines and are mimicked by several N^6-substituted adenosine analogs, including all of the N^6-modified compounds used in this study. In contrast to the potent inhibitory effects of these analogs, ribose-modified analogs of adenosine are only weakly effective on the intact fat cell. Yet when these investigators examined adenylate cyclase activity in isolated membrane preparations, they found only relatively weak inhibition by adenosine, no effects of the N^6-substituted analogs, no effects of methylxanthines, and potent inhibition by some ribose-modified analogs. It seems apparent that, because of

inadequate GTP concentration in the assay medium, only P site-mediated inhibition was observed in the previously published studies (18–21). Inhibition by R site receptors for adenosine requires the presence of GTP concentrations sufficient to react with the inhibitory nucleotide process (see below); only then can one demonstrate, with isolated membranes, direct and potent effects of purine-modified analogs on adenylate cyclase activity that are reversed by methylxanthines. Thus, we suggest that GTP is required in the intact fat cell for R site-mediated inhibition of cyclic AMP production and lipolysis. The fact that "inhibitory" levels of GTP (10 nM–1 μM) were required for R site action in the isolated membranes raises the possibility that adenylate cyclase in the intact cell is in a GTP-inhibited state.

One of the earliest observations with the isolated adipocyte preparations was that methylxanthines were required in order to observe hormone-stimulated increases in cyclic AMP production and lipolysis (34). Although methylxanthines can inhibit cyclic AMP breakdown by phosphodiesterases, it has been suggested that the major action of these compounds on fat cells is the antagonism of the inhibitory effects of adenosine (20–22). Our data showing that methylxanthines act as blockers at the R site provide direct evidence that these compounds can increase cyclic AMP production and lipolysis in the fat cell through a direct action on the adenylate cyclase system.

There is convincing evidence that, in those cells in which R site effectors increase cyclic AMP formation, they do so by reacting with cell surface receptors (5, 7, 13, 15, 16). In this sense, hormones and adenosine are similar in that their actions require intervention within the membrane of a process that couples receptors to the catalytic component of the cyclase. The analogy between hormones and adenosine action on adenylate cyclase is strengthened by the finding that both agents require GTP for activation of the enzyme *in vitro*. The GTP dependence for hormone action is well documented (35). Clark and Seney (11) and Wolff and Cook (15) have observed synergism between adenosine and guanine nucleotides in activation of adenylate cyclase in cultured cells. We have made a similar observation with human platelet adenylate cyclase (unpublished data). The analogy between adenosine and hormone actions is particularly evident with the turkey erythrocyte enzyme, with which either adenosine or catecholamine plus 5′-guanylylimidodiphosphate produces a persistently activated adenylate cyclase (36). As in those cases in which adenosine increases cellular cyclic AMP production, the available evidence indicates that R site-reactive analogs and low concentrations of adenosine inhibit adipocyte cyclic AMP production by interacting with a cell surface receptor (19, 22, 30). In this report we have demonstrated a GTP dependence for adenosine inhibition of the fat cell cyclase, a finding that further emphasizes the central role of guanine nucleotides in mediating signals from the cell surface receptors to the catalytic component of the cyclase. In the adipocyte system, R site effectors plus GTP inhibit adenylate cyclase activity, whereas in other systems this combination stimulates it.

The GTP-dependent process that facilitates activation of fat cell cyclase by hormones such as catecholamines and corticotropin appears to be functionally distinct from the GTP-dependent inhibitory process (26), and it is the latter that mediates inhibition by adenosine R site analogs. Thus, our data suggest that one ligand (adenosine), acting at a cell surface receptor (R site), can inhibit through a GTP-mediated process the action by another ligand (catecholamine, corticotropin) that also acts at a cell surface receptor to stimulate through a second GTP-mediated process.

Inhibition of adenylate cyclases by low concentrations of guanine nucleotides is not unique to the fat cell. Birnbaumer *et al.* (37) have reported inhibition of basal and hormone-stimulated renal medullary cyclase. The GTP analog, 5′-guanylylimidophosphate, which strongly activates eukaryotic adenylate cyclases (38), inhibits the enzyme from cardiac sarcolemma under certain conditions (39). In two other systems, inhibition by GTP has provided a means for duplicating in membranes the effects of agents on intact cells. First, Jacobs (40) has shown that α-adrenergic inhibition of human platelet adenylate cyclase is dependent on GTP. Second, Tell *et al.* (41) found that, in order to elicit activation of caudate nucleus cyclase with dopamine, the enzyme first had to be inhibited with GTP; dopamine then reversed this inhibition. These examples, plus the data in this paper, suggest that GTP-mediated inhibition of adenylate cyclases may be of physiologic importance. Preliminary experiments with several ligands known to depress cyclic AMP levels in adipocytes, such as nicotinic acid, prostaglandins, and insulin (42), indicate that they too inhibit adenylate cyclase in a GTP-dependent manner.

1. Londos, C. & Wolff, J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5482–5486.
2. Sattin, A. & Rall, T. W. (1970) *Mol. Pharmacol.* **6**, 13–23.
3. Huang, M., Shimizu, H. & Daly, J. W. (1972) *J. Med. Chem.* **5**, 462–466.
4. Haslam, R. J. & Lynham, J. A. (1972) *Life Sci.* **11**, 1143–1154.
5. Clark, R. B., Gross, R., Su, Y. F. & Perkins, J. P. (1974) *J. Biol. Chem.* **249**, 5296–5303.
6. Peck, W. A., Carpenter, J. & Messinger, K. (1974) *Endocrinology* **94**, 148–154.
7. Blume, A. J. & Foster, C. J. (1975) *J. Biol. Chem.* **250**, 5003–5008.
8. Haslam, R. J. & Rosson, G. M. (1975) *Mol. Pharmacol.* **11**, 528–544.
9. Maguire, M. E., Sturgill, T. W., Anderson, H. S., Minna, J. D. & Gilman, A. G. (1975) *Adv. Cyclic Nucleotide Res.* **5**, 699–718.
10. Wilkening, D. & Makman, M. H. (1975) *Brain Res.* **92**, 522–528.
11. Clark, R. B. & Seney, M. N. (1976) *J. Biol. Chem.* **251**, 4239–4246.
12. Iizuka, H., Adachi, K., Halprin, K. M. & Levine, U. (1976) *Biochim. Biophys. Acta* **444**, 685–693.
13. Huang, M. & Drummond, G. (1976) *Biochem. Pharmacol.* **25**, 2713–2719.
14. Penit, J., Huot, J. & Jard, S. (1976) *J. Neurochem.* **26**, 265–273.
15. Wolff, J. & Cook, G. H. (1976) *J. Biol. Chem.* **252**, 687–693.
16. Green, R. D. & Stanberry, L. R. (1977) *Biochem. Pharmacol.* **26**, 37–43.
17. Premont, R., Perez, M. & Bockaert, J. (1977) *Mol. Pharmacol.* **13**, 662–670.
18. Fain, J. N., Pointer, R. H. & Ward, W. F. (1972) *J. Biol. Chem.* **247**, 6866–6872.
19. Fain, J. N. (1973) *Mol. Pharmacol.* **9**, 595–604.
20. Fain, J. N. & Weiser, P. B. (1975) *J. Biol. Chem.* **250**, 1027–1034.
21. Trost, T. & Stock, K. (1977) *Arch. Pharmacol.* **299**, 33–40.
22. Schwabe, U. & Ebert, R. (1974) *Arch. Pharmacol.* **282**, 33–44.
23. Harwood, J. P., Low, H. & Rodbell, M. (1973) *J. Biol. Chem.* **248**, 6239–6245.
24. Rodbell, M. (1975) *J. Biol. Chem.* **250**, 5826–5834.
25. Yamamura, H., Rodbell, M. & Fain, J. N. (1976) *Mol. Pharmacol.* **12**, 693–700.
26. Yamamura, H., Lad, P. M. & Rodbell, M. (1977) *J. Biol. Chem.* **252**, 7964–7966.
27. Salomon, Y., Londos, C. & Rodbell, M. (1974) *Anal. Biochem.* **58**, 541–548.

28. Kimura, N., Nakane, K. & Nagata, N. (1976) *Biochem. Biophys. Res. Commun.* **70**, 1250–1256.
29. Avruch, J. & Wallach, D. F. H. (1971) *Biochim. Biophys. Acta* **233**, 334–337.
30. Westermann, E. & Stock, K. (1970) in *Adipose Tissue: Regulation and Metabolic Functions*, eds., Jeanrenaud, B. & Hepp, D. (G. Thieme, Stuttgart, Germany), pp. 47–54.
31. Schild, H. O. (1949) *Br. J. Pharmacol.* **4**, 277–280.
32. Ebert, R. & Schwabe, U. (1972) *Arch. Pharmacol.* **278**, 247–259.
33. Schwabe, U., Ebert, R. & Erbler, H. C. (1973) *Arch. Pharmacol.* **276**, 133–148.
34. Butcher, R. W., Baird, C. E. & Sutherland, E. W. (1968) *J. Biol. Chem.* **243**, 1705–1712.
35. Rodbell, M., Lin, M. C., Salomon, Y., Londos, C., Harwood, J. P., Martin, B. R., Rendell, M. & Berman, M. (1975) *Adv. Cyclic Nucleotide Res.* **5**, 3–29.
36. Sevilla, N., Tolkovsky, A. M. & Levitzki, A. (1977) *FEBS Lett.* **81**, 339–341.
37. Birnbaumer, L., Nakahara, T. & Yang, P. C. (1974) *J. Biol. Chem.* **249**, 7857–7866.
38. Londos, C., Salomon, Y., Lin, M. C., Harwood, J. P., Schramm, M., Wolff, J. & Rodbell, M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3087–3090.
39. Narayanan, N. & Sulakhe, P. V. (1978) *Arch. Biochem. Biophys.* **185**, 72–81.
40. Jacobs, K. H. (1978) in *Molecular Biology and Pharmacology of Cyclic Nucleotides*, eds., Folco, G. & Paoletti, R. (Elsevier, Amsterdam), pp. 265–278.
41. Tell, G. P., Pasternak, G. W. & Cuatrecasas, P. (1975) *FEBS Lett.* **51**, 242–245.
42. Fain, J. N. (1973) *Pharmacol. Rev.* **25**, 67–118.