ally secreted calcium carbonate. Favositids can be recognized therefore as an order of Demospongiae. They originated in the early Phanerozoic, probably from a lithistid stock of demosponges. This presumably happened during the Ordovician when some lithistid invaded trophically attractive but calcium oversaturated areas of the epicontinental seas (*21*). In response to high calcium stress they developed calicoblasts within the pinacoderm, thus neutralizing the toxic calcium excess by secreting it as calcium carbonate. Such a suggestion seems to be supported by observations (*22*) made on the recent sclerosponge *Merlia normani* Kirkpatrick, which secretes a calcareous skeleton that may be absent in specimens growing in presumably less calcium saturated waters. In that respect sclerosponges appear to be interesting model organisms in studies on calcareous biomineralization and on the origin of calcareous hard parts in general (*23*).

The adaptive evolutionary trend to secrete a basal calcareous skeleton was repeated several times in geological history by various groups of demosponges producing highly homeomorphic calcareous structures that differ strongly in spicule character (for example, among massive sclerosponges: *Acanthochaetetes, Tabulospongia, Favosites* s.l., *Merlia*, and *Chaetetopsis*; among arborescent sclerosponges: *Thamnopora* and *Neuropora*). Reversed trends resulting in sclerosponge taxa with almost identical spicule character but differing significantly in the morphology of the calcareous skeleton are also known (for example, *Hispidopetra, Boswellia, Chaetetopsis*). Both phenomena make the taxonomic treatment of fossil sclerosponges in which spicules are only exceptionally preserved extremely difficult. This may account for the enormously complicated taxonomy of favositids, consisting of hundreds of poorly defined species and genera. The discovery of further spiculated favositids will help in establishing a sound classification and phylogeny of the group.

The discovery of spicules in the favositids does not imply that other, less abundant groups of Tabulata are sclerosponges. Nevertheless, reexamination of such tabulate "corals" as halysitids, syringoporids, and several related groups may throw new light on their affinities, particularly if collected from strongly leached horizons.

JÓZEF KAŹMIERCZAK
Polish Academy of Sciences,
Institute of Paleobiology,
Al. Zwirki i Wigury 93,
PL-02089 Warszawa, Poland

24 AUGUST 1984

References and Notes

1. W. D. Hartman, *Postilla 137* (1969).
2. _____ and T. F. Goreau, *Symp. Zool. Soc. London* **25**, 205 (1970).
3. _____, *Postilla 167* (1975).
4. K. Mori, *Tohoku Univ. Sci. Rep. Ser. 2 Geol.* **46**, 1 (1976); *ibid.* **47**, 1 (1977).
5. R. Kirkpatrick, *Nature (London)* **89**, 502 (1912); *ibid.*, p. 607.
6. D. I. Gray, *Palaeontology* **23**, 803 (1981).
7. G. Dieci, F. Russo, M. S. Marchi, *Boll. Sci. Paleontol. Ital.* **16**, 229 (1977); J. Kaźmierczak, *Neues Jahrb. Geol. Palaeontol. Monatsh.* **2**, 97 (1979).
8. J. Kaźmierczak, *Nature (London)* **264**, 49 (1976); *Acta Palaeontol. Pol.* **25**, 243 (1980); _____ and W. E. Krumbein, *Lethaia*, **16**, 207 (1983).
9. W. D. Hartman, *Colloq. Int. C.N.R.S.* **291**, 467 (1979).
10. K. Brood, *Geol. Foeren. Stockholm Foerh.* **94**, 393 (1972).
11. H. W. Flügel, *Lethaia* **9**, 405 (1976); J. H. Stel, *Studies on the Palaeobiology of Favositids* (Stabo, Groningen, 1978).
12. A. von Schouppé and K. Oekentorp, *Palaeontographica Abt. A* **145**, 79 (1974); W. A. Oliver, Jr., *Paleobiology* **5**, 188 (1979).
13. The specimen was recovered during reexamination of the undescribed collection of Devonian tabulates from the Holy Cross Mountains at the Institute of Paleobiology, Polish Academy of Sciences in Warsaw. It is represented only by one transverse thin section labeled originally 27 and stored now under catalog number ZPAL T.XV/1. For description and illustrations of other *Th. reticulata* specimens from the same collecting site see A. Stasińska, *Acta Palaeontol. Pol.* **3**, 16 (1958).
14. For detailed geographical and geological data of the Skalska Series, see M. Pajchlowa, *Biul. Inst. Geol. Warsaw* **122**, 145 (1957).
15. D. Hill and E. C. Stumm, in *Treatise on Invertebrate Paleontology, Coelenterata*, R. C. Moore, Ed. (Univ. of Kansas Press, Lawrence, 1956), part F, p. F444.
16. M. W. de Laubenfels, in *Treatise on Invertebrate Paleontology, Porifera*, R. C. Moore, Ed. (Univ. of Kansas Press, Lawrence, 1955), part E, p. E21; W. D. Hartman, J. W. Wendt, F. Wiedenmayer, *Sedimenta* **VIII**, 55 (1980).
17. L. F. Laporte, Ed., *Soc. Econ. Paleontol. Mineral. Spec. Publ. 18* (1974); D. F. Toomey, Ed., *ibid. 30* (1981).
18. M. Kato, *J. Fac. Sci. Hokkaido Univ. Ser. 4, Geol. Mineral.* **14**, 51 (1968); K. Oekentorp, *Muenstersche Forsch. Geol. Palaeontol.* **24**, 35 (1972).
19. F. M. Bayer, in *Treatise on Invertebrate Paleontology, Coelenterata*, R. C. Moore, Ed. (Univ. of Kansas Press, Lawrence, 1956), part F, p. F166.
20. C. Lévi, in *Traité de Zoologie*, P.-P. Grassé, Ed. (Masson, Paris, 1973), vol. 3, fasc. 1, p. 577; J. Vacelet, *Bull. Mus. Nat. Hist. Sér. 444 Zool.* **307**, 345 (1977); _____, P. Vasseur, C. Lévi, *Mem. Mus. Nat. Hist. Nat. A44* (1976).
21. E. T. Degens, J. Kaźmierczak, V. Ittekkot, *J. Geophys. Res.*, in press.
22. L. Pouliquen, *Tethys* **3**, 717 (1972).
23. J. Kaźmierczak, V. Ittekkot, E. T. Degens, in preparation.
24. I thank E. T. Degens and V. Ittekkot (Hamburg) for stimulating discussions and C. R. Jones (Leicester) for linguistic help. Supported by the Polish Academy of Sciences and the Alexander von Humboldt Foundation.

17 October 1983; 17 May 1984

Direct Demonstration of Impaired Functionality of a Purified Desensitized β-Adrenergic Receptor in a Reconstituted System

Abstract. *Long-term exposure of various cell types to β-adrenergic agonists such as isoproterenol leads to an attenuated responsiveness ("desensitization") of the adenylate cyclase system to further challenge with these agonists. The turkey erythrocyte model system was used earlier to show that a covalent modification of the receptor (phosphorylation) is associated with this process. The functionality of the "desensitized" β-adrenergic receptor was assessed by implanting purified β-adrenergic receptor preparations from control and desensitized turkey erythrocytes into phospholipid mixtures and then fusing them with receptor-deficient cells (*Xenopus laevis *erythrocytes). Desensitized β-adrenergic receptors showed a 40 to 50 percent reduction in their ability to couple to the heterologous adenylate cyclase system, comparable to the reduction in their functionality observed in their original membrane environment. These results demonstrate the utility of recently developed receptor reconstitution techniques for assessing the functionality of purified receptors and show a direct link between a covalent modification of a membrane-bound receptor and its impaired functionality in a reconstituted system.*

A general property of many eukaryotic cells is an adaptive process whereby the cell becomes progressively less responsive to a hormone or drug with time. Such processes have been variably referred to as "desensitization," "tolerance," or "tachyphylaxis" and have been reported to occur in systems in which the biological responses are mediated by adenosine 3′,5′-monophosphate (cyclic AMP) (*1*), as well as in those in which the hormonal responses are independent of this cyclic nucleotide (*2*). Among the hormone-responsive adenylate cyclase systems, the most widely studied example has been the adenylate cyclase-coupled β-adrenergic receptor, which mediates the stimulatory effects of catecholamines on physiological functions in heart, smooth muscle, and other tissues (*3*). This system consists of at least three components: the β-adrenergic receptor–binding protein, which binds hormones and triggers the biological response; the nucleotide regulatory protein (N or G/F), which binds guanine nucleotides; and the catalytic moiety of the adenylate cyclase, to which the guanine nucleotide protein binds, thereby coupling the agonist-receptor complex to the effector molecules (*4*).

Although the detailed mechanisms of hormone-promoted desensitization remain to be unraveled, in the β-adrenergic

receptor–coupled adenylate cyclase system, evidence points to the receptors themselves as a major locus of alteration. There appear to be several mechanisms for altering receptor function. In some instances—for example, frog erythrocytes and various cultured mammalian cell lines (*5–7*)—a functionally normal receptor may be sequestered away from the effector adenylate cyclase complex by translocation to an intracellular membrane compartment (*8*). In contrast, in certain other systems such as avian erythrocytes (turkey and pigeon) (*9, 10*), the desensitization of the adenylate cyclase is associated with a structural alteration in the receptor which is apparent as an altered mobility on sodium dodecyl sulfate (SDS) gels of the photoaffinity-labeled receptor (*11*). This structural alteration correlates with an increased state of phosphorylation of the desensitized β-adrenergic receptor (*12*). It has not, however, been possible to assess whether the structural alterations in the desensitized receptors are directly related to the impaired functionality of these macromolecules as reflected in the desensitized state of the adenylate cyclase system of the cell. Recently, however, new techniques have been developed for investigating the molecular properties of the receptors.

β-Adrenergic receptor–binding proteins can be solubilized in digitonin and purified by affinity chromatography (*13*). The structure of the receptors can be studied with the use of high-affinity, specific photoaffinity probes such as ^{125}I-labeled *p*-azidobenzylcarazolol (^{125}I-pABC) (*14*). The receptors, purified in digitonin, can be implanted into phospholipid vesicles and their functionality assessed by fusing them to *Xenopus laevis* erythrocytes that contain both the N protein and the catalytic subunit of the adenylate cyclase system but which lack β-adrenergic receptors (*8, 15, 16*). Using these techniques, we have demonstrated the bifunctionality of the isolated β-adrenergic receptor proteins—that is, these macromolecules both bind β-adrenergic hormones and confer sensitivity to catecholamine stimulation on the adenylate cyclase system of a heterologous cell (*15, 16*).

We used these techniques to assess the functional capability of a β-adrenergic receptor derived from a desensitized cell. For this purpose, we used affinity chromatography–purified receptor preparations from control and desensitized turkey erythrocytes. Since the turkey erythrocyte β-adrenergic receptors are of the β_1 subtype, we first sought to determine whether the specificity of the catecholamine responsiveness conferred on the acceptor *X. laevis* erythrocytes by the purified receptors would be conserved. Figure 1, A and B, shows catecholamine dose-response curves for the stimulation of adenylate cyclase in native turkey erythrocyte membranes and in the *X. laevis* erythrocyte membranes after fusion with the receptor-containing vesicles—before fusion there is no catecholamine-responsive adenylate cyclase in these membranes (*8*). The order of potency and the affinity of the β-adrenergic agonists isoproterenol, epinephrine, and norepinephrine for stimulation of adenylate cyclase activity are essentially the same in the reconstituted system as in the native turkey erythrocyte membranes (Fig. 1, A and B). The potency series in both cases is isoproterenol > epinephrine ≥ norepinephrine, which is typical for a β_1 receptor. The findings indicate that the specificity and subtype specificity of the purified receptor is conserved through the reconstitution-fusion procedures and indicate the high fidelity of these procedures for assessing receptor functionality.

Incubation of turkey erythrocytes with

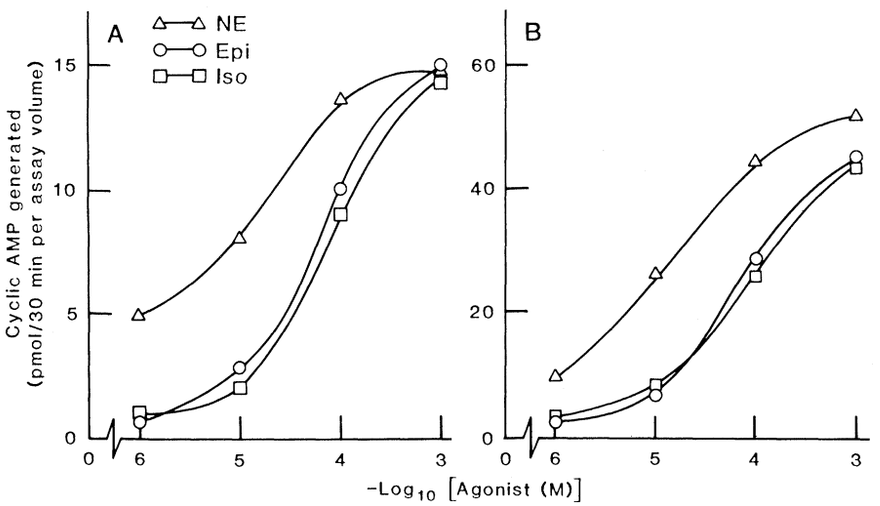

Fig. 1. (A) Dose-response curves for agonist stimulation of adenylate cyclase in crude membranes of control turkey erythrocytes. Cells were washed three times with 150 m*M* NaCl, 10 m*M* dextrose, 0.2 m*M* sodium metabisulfite, and 17 m*M* tris-HCl (*p*H 7.4) and hypotonically lysed with 5 m*M* $MgCl_2$ and 5 m*M* tris HCl (*p*H 7.4). The lysates were centrifuged at 1000 rev/min for 10 minutes and the pellets containing the nuclear material, unlysed cells, and other cell debris were discarded. The supernatant containing the plasma membranes was centrifuged at 40,000*g* for 10 minutes. After four additional washes with 5 m*M* $MgCl_2$ and 75 m*M* tris-HCl (*p*H 7.4), the pellets were resuspended in "adenylate cyclase buffer" containing 75 m*M* tris HCl (*p*H 7.4), 12.5 m*M* $MgCl_2$, 1.5 m*M* EDTA, 250 m*M* sucrose, and 2 m*M* dithiothreitol at a concentration of 8 mg of protein per milliliter of buffer. The adenylate cyclase assay was performed as described (*17, 18*) in the presence of 0.1 m*M* guanosine triphosphate (GTP) and 0.1 m*M* adenosine triphosphate (ATP). Results were reported as picomoles of cyclic AMP generated per assay volume in 30 minutes at 30°C. (B) Dose-response curves for agonist stimulation of adenylate cyclase in fused hybrids of reconstituted affinity chromatography–purified control turkey β-adrenergic receptor preparations and *X. laevis* erythrocytes. Purified turkey erythrocyte membranes were prepared as described (*13*). The β-adrenergic receptor was solubilized from erythrocyte plasma membranes in a buffer containing 1 percent digitonin and purified 500- to 1000-fold by affinity chromatography on a Sepharose-alprenolol gel (*13, 15*). The column elution buffer contained 0.025 percent digitonin, 100 m*M* NaCl, and 10 m*M* tris-HCl (*p*H 7.4). Purified receptor preparations were reconstituted into vesicles containing a mixture of soybean phosphatidylcholine and a light membrane vesicle fraction of frog erythrocyte lipids as described earlier (*16*), except that the detergent was removed from the incubation mixture by elution through an Extracti-gel (Pierce) column, rather than by treatment with SM-2 resin. The eluates were diluted with 15 ml of 100 m*M* NaCl and 10 m*M* tris-HCl (*p*H 7.4) and centrifuged at 250,000*g* for 1.5 hours. The fusion of the reconstituted β-adrenergic receptors with *X. laevis* erythrocytes was performed exactly as described (*15*). Pellets containing the reconstituted β-adrenergic receptor were mixed with 2×10^7 packed *X. laevis* erythrocytes. The mixture was then incubated with 15 μl of phospholipids (200 μg of soybean phosphatidylcholine and 10 μg of lysophosphatidylcholine) for 5 minutes at 5° to 10°C and then with 10 μl of 10 m*M* $MgCl_2$ for 5 minutes. Fusion was performed with polyethyleneglycol (50 percent, weight to volume) at 30°C (*8, 15, 16, 19*). Adenylate cyclase activity in the resulting hybrid cell membranes was measured in membranes prepared by freeze-thaw lysis. The assay was performed in the presence of 0.1 m*M* GTP and 0.1 m*M* ATP (*8, 15, 16*). Each set of data shown represents the means of triplicate determinations from representative experiments repeated twice with the comparable results. Abbreviations: Iso, isoproterenol; Epi, epinephrine; and NE, norepinephrine.

$10^{-5}M$ isoproterenol for 2.5 hours leads to 50 to 60 percent inhibition of isoproterenol-stimulated adenylate cyclase activity in membranes prepared from these cells (Fig. 2A). In agreement with previous findings (*11*), after desensitization of the turkey erythrocytes, the β-adrenergic receptor polypeptides specifically labeled with ^{125}I-pABC in the membranes were of larger apparent molecular weight than the controls (41,000 versus 36,000 for the major band and 53,000 versus 50,000 for the minor band). This altered mobility of the receptor peptides from desensitized cells appears to correlate with their increased phosphorylation (*12*).

We wished to assess directly the functionality of these desensitized β-adrenergic receptors and to establish whether their covalent modification during desensitization is associated with an altered ability to couple to the other elements of the adenylate cyclase system. This was accomplished by purifying the receptors from control and desensitized turkey erythrocytes by affinity chromatography (500- to 1000-fold), implanting them into phospholipid vesicles, and fusing them with *X. laevis* erythrocytes. Fusion of 1 pmol of reconstituted β-adrenergic receptor prepared from control turkey erythrocytes established a sevenfold isoproterenol stimulation of adenylate cyclase activity in the *X. laevis* erythrocyte, which was previously unresponsive to catecholamines (Fig. 2B). By contrast, the same amount of purified reconstituted β-adrenergic receptor from desensitized turkey erythrocytes established a diminished responsiveness to catecholamines in the heterologous adenylate cyclase system, conferring upon it only a fourfold stimulation by isoproterenol. This represents a 45 percent reduction in the reconstituted response ($P < 0.05$), which correlates quite well with the extent of isoproterenol-induced desensitization of the adenylate cyclase in the crude membranes (Fig. 2A). The altered mobility of the affinity-purified receptor on gel electrophoresis is preserved and is comparable to that observed in crude membranes (compare insets in Fig. 2, A and B). The stability of this structural modification of the receptor induced during desensitization presumably underlies our ability to observe the diminished functionality of the desensitized receptor even after purification, reconstitution, and fusion.

Our data demonstrate a direct relation between a stable modification of a receptor due to hormone-induced desensitization and an impairment in its function assessed in a reconstituted system. These data do not resolve the issue of whether all of the desensitized receptors are partially impaired in their function,

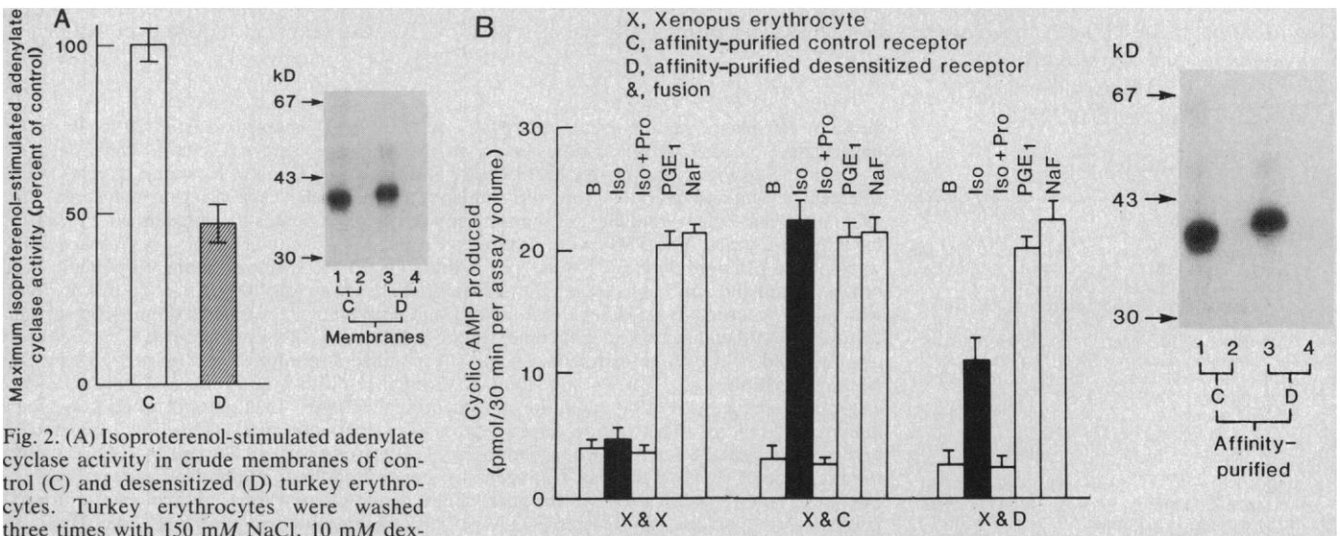

Fig. 2. (A) Isoproterenol-stimulated adenylate cyclase activity in crude membranes of control (C) and desensitized (D) turkey erythrocytes. Turkey erythrocytes were washed three times with 150 m*M* NaCl, 10 m*M* dextrose, 0.2 m*M* sodium metabisulfite, and 17 m*M* tris-HCl (*p*H 7.4) and incubated with buffer only (control) or buffer containing $10^{-5}M$ isoproterenol (desensitized) for 2.5 hours. Cells were hypotonically lysed, and the membranes were prepared as described in the legend to Fig. 1A. The isoproterenol-stimulated adenylate cyclase of the control membranes was 250 ± 16 pmol per milligram of protein in 30 minutes, whereas the adenylate cyclase in the desensitized membranes was 117 ± 12 pmol/mg in 30 minutes. Each set of data represents the means ± standard error of the mean triplicate determinations from five different experiments. (Inset) Autoradiogram of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of ^{125}I-pABC–labeled β-adrenergic receptors from crude membranes prepared from control and desensitized turkey erythrocytes. Plasma membranes from control and desensitized erythrocytes were incubated with ^{125}I-pABC in the presence and absence of $10^{-5}M$ (−)-alprenolol and photolysed as described earlier (*6, 14*). The samples were: (lane 1) ^{125}I-pABC-labeled membranes from control cells, (lane 2) membranes from control cells labeled with ^{125}I-pABC in the presence of $10^{-5}M$ (−)-alprenolol, (lane 3) ^{125}I-pABC-labeled membranes from desensitized cells, and (lane 4) membranes from desensitized cells labeled with ^{125}I-pABC in the presence of $10^{-5}M$ (−)-alprenolol. The SDS-PAGE was performed by the method of Laemmli (*20*). Proteins labeled with ^{125}I-pABC were detected by autoradiography for 1 to 3 days in the presence of intensifying screens. (B) Fusion of *X. laevis* erythrocytes with affinity chromatography–purified and reconstituted β-adrenergic receptors from control and desensitized turkey erythrocytes. Affinity-purified receptor preparations were reconstituted into a mixture of soybean phosphatidylcholine and a light membrane fraction of frog erythrocyte lipids (*15*) (see also legend to Fig. 1B). The same number of control and desensitized receptors were used in the reconstitution protocol, and the same efficiency of reconstitution into the lipid vesicles (20 to 25 percent) was observed in both cases. The fusion with *X. laevis* erythrocytes was performed as described earlier (*8, 15, 16*) and in the legend to Fig. 1B. Each set of data shown in the individual panels represents the means ± standard error of the mean of triplicate determinations from four experiments. Abbreviations: B, basal; Iso, $5 \times 10^{-5}M$ (−)-isoproterenol; Iso + Pro, $5 \times 10^{-5}M$ (−)-isoproterenol plus $5 \times 10^{-5}M$ (±)-propranolol; PGE_1, $3 \times 10^{-6}M$ prostaglandin E_1; and NaF, 10 m*M* NaF. (Inset) Autoradiogram of SDS-PAGE of ^{125}I-pABC photoaffinity-labeled, purified β-adrenergic receptors from control and desensitized turkey erythrocytes used in these experiments. Purified β-adrenergic receptor samples were incubated with 30 p*M* ^{125}I-pABC for 60 minutes at 25°C, desalted on Sephadex G-50 columns, and photolysed for 90 seconds as described earlier (*13*). The samples were: (lane 1) ^{125}I-pABC-labeled, purified control β-adrenergic receptors (C), (lane 2) purified control β-adrenergic receptors labeled in the presence of $10^{-5}M$ (−)-alprenolol, (lane 3) ^{125}I-pABC labeled purified desensitized β-adrenergic receptors (D), and (lane 4) purified desensitized β-adrenergic receptors photoaffinity labeled in the presence of $10^{-5}M$ (−)-alprenolol. The SDS-PAGE was performed as described (*20*). The experiment was repeated three times with comparable results.

or whether about half of them have completely lost their ability to stimulate adenylate cyclase. They do demonstrate that the receptors are the locus of the alteration in this system.

The methods described here open the way to exploring the fundamental biochemical mechanisms of these processes. Thus, it should be possible to attempt to mimic the functional alterations of the receptors by directly modifying their structure—for example, using appropriate kinases to phosphorylate the purified receptors in vitro. The use of these methods to assess the biological activating function of receptors can help to elucidate the molecular mechanisms that regulate receptor activity. These methods provide a major advance in attempts to understand receptor function and its regulation.

BERTA STRULOVICI
RICHARD A. CERIONE
BRIAN F. KILPATRICK
MARC G. CARON
ROBERT J. LEFKOWITZ
Howard Hughes Medical Institute, Departments of Medicine (Cardiology), Biochemistry, and *Physiology, Duke University Medical Center, Durham, North Carolina 27710*

References

1. R. J. Lefkowitz, M. R. Wessels, J. M. Stadel, *Curr. Top. Cell Regul.* **17**, 205 (1980); T. K. Harden, *Pharmacol. Rev.* **35**, 5 (1983).
2. J. R. Gavin, J. Roth, D. M. Neville, P. De Meyts, D. N. Buell, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 84 (1974).
3. E. W. Sutherland, G. A. Robison, R. W. Butcher, *Circulation* **37**, 279 (1968).
4. E. M. Ross, S. E. Pedersen, V. A. Florio, *Curr. Top. Membr. Transp.* **18**, 109 (1982); R. J. Lefkowitz, J. M. Stadel, M. G. Caron, *Annu. Rev. Biochem.* **52**, 159 (1983).
5. De-M. Chuang and E. Costa, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3024 (1979).
6. J. M. Stadel *et al.*, *J. Biol. Chem.* **258**, 3032 (1983).
7. T. K. Harden, C. U. Cotton, G. L. Waldo, J. K. Lutton, J. P. Perkins, *Science* **210**, 441 (1980).
8. B. Strulovici, J. M. Stadel, R. J. Lefkowitz, *J. Biol. Chem.* **258**, 6410 (1983).
9. B. B. Hoffman, D. Mullikin-Kilpatrick, R. J. Lefkowitz, *J. Cyclic Nucleotide Res.* **5**, 355 (1979).
10. T. H. Hudson and G. L. Johnson, *Mol. Pharmacol.* **20**, 694 (1981).
11. J. M. Stadel, P. Nambi, T. N. Lavin, S. L. Heald, M. G. Caron, R. J. Lefkowitz, *J. Biol. Chem.* **257**, 9292 (1982).
12. J. M. Stadel, P. Nambi, R. G. L. Shorr, D. F. Sawyer, M. G. Caron, R. J. Lefkowitz, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3173 (1983).
13. R. G. L. Shorr, M. W. Strohsacker, T. N. Lavin, R. J. Lefkowitz, M. G. Caron, *J. Biol. Chem.* **257**, 12341 (1982).
14. T. N. Lavin, P. Nambi, S. L. Heald, P. W. Jeffs, R. J. Lefkowitz, M. G. Caron, *ibid.*, p. 12332.
15. R. A. Cerione, B. Strulovici, J. L. Benovic, C. D. Strader, M. G. Caron, R. J. Lefkowitz, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4899 (1983).
16. R. A. Cerione, B. Strulovici, J. L. Benovic, R. J. Lefkowitz, M. G. Caron, *Nature (London)* **306**, 566 (1983).
17. R. J. Lefkowitz, *J. Biol. Chem.* **249**, 6119 (1974).
18. Y. Salomon, D. Londos, M. Rodbell, *Anal. Biochem.* **58**, 541 (1974).
19. M. Schramm, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1174 (1979).
20. U. K. Laemmli, *Nature (London)* **277**, 680 (1970).

20 January 1984; accepted 22 May 1984

Isolation of Lymphocytopathic Retroviruses from San Francisco Patients with AIDS

Abstract. *Infectious retroviruses have been detected in 22 of 45 randomly selected patients with acquired immune deficiency syndrome (AIDS) and in other individuals from San Francisco. The AIDS-associated retroviruses (ARV) studied in detail had a type D morphology, Mg^{2+}-dependent reverse transcriptase, and cytopathic effects on lymphocytes. The viruses can be propagated in an established adult human T cell line, HUT-78. They cross-react with antiserum to the lymphadenopathy-associated retrovirus isolated from AIDS patients in France. Antibodies to ARV were found in all 86 AIDS patients and in a high percentage of 88 other homosexual men in San Francisco. This observation indicates the widespread presence of these lymphocytopathic retroviruses and their close association with AIDS.*

Acquired immune deficiency syndrome (AIDS) has affected more than 4000 individuals in the world; in San Francisco, over 600 cases have been reported (*1*). In addition, there are many patients with unexplained chronic lymphadenopathy which may be caused by the agent responsible for AIDS (*1*). Last year, two different retroviruses were isolated from AIDS patients. One of these, human T cell leukemia virus (HTLV-I) (*2*), which is associated with T cell leukemias in man (*3*), has a type C morphology as determined by electron microscopy; this virus can immortalize T cells to produce continuous cell lines and is primarily cell-associated (*3*). The other, lymphadenopathy-associated retrovirus (LAV), was isolated from the lymph node of a patient with lymphadenopathy (*4*) and has subsequently been recovered from patients with AIDS (*5*). LAV has a type D morphology, causes cytopathic changes in T cells, and is infectious in culture fluids. A third retrovirus, HTLV-III, was recently reported in AIDS pa-

Table 1. Peripheral mononuclear cell (PMC) cultures were established from 10 to 30 ml of heparinized blood from individuals seen at the Kaposi's sarcoma clinic, University of California, San Francisco, or the AIDS clinic, San Francisco General Hospital. Patients were selected at random intervals from the sequence of individuals appearing at the clinics for evaluation. All patients and most of the clinically healthy individuals had lived in San Francisco for at least 2 years. The PMC were separated on Ficoll-Hypaque gradients (*9*). Washed cells were plated at approximately 2×10^6 per milliliter in RPMI 1640 containing 10 percent fetal bovine serum and antibiotics (penicillin, 100 unit/ml; streptomycin 100 μg/ml). To this medium was added interleukin-2 (Meloy) (0.5 μg/ml) and Polybrene (1 μg/ml). At initiation of the cultures, phytohemagglutinin (Wellcome), approximately 2.5 μg/ml, was added. Some cultures also received $10^{-5}M$ β-mercaptoethanol and sheep antisera to interferon-α (*4*) provided by the National Institutes of Health (lot 61220); K. Cantell, Helsinki; or F. Barré-Sinoussi, Paris. These antisera were used at a dilution that neutralized 700 to 1000 units of interferon-α per milliliter of culture. The culture supernatants were routinely assayed for Mg^{2+}-dependent reverse transcriptase activity (see legend to Fig. 1) every 3 to 6 days. The cells were studied for the presence of HTLV-I and LAV antigens by standard indirect immunofluorescence assays. For these studies, cells were put on glass slides, air dried, and fixed in cold acetone for 15 minutes. A monoclonal antibody to HTLV p19 provided by Robert-Guroff and Gallo, National Institutes of Health, and a monoclonal antibody to adult T cell leukemia virus (ATLV) p19/p28 provided by Y. Hinuma, Kyoto, Japan (*10*), were used. For detection of LAV, human serum (from patient B.R.U.) provided by Barré-Sinoussi, Paris, was used (*4*). Peripheral mononuclear cells producing ARV were also tested for reactivity with sera from AIDS patients from San Francisco. For the sera we examined, the results were the same as those obtained with the B.R.U. serum. The fluorescein-conjugated antibodies for these immunofluorescence assays were either goat antiserum to mouse immunoglobulin G or goat antiserum to human immunoglobulin G. Some cultures were examined for virus by electron microscopy (see legend to Fig. 2). Any cultures that gave positive results repeatedly by any of these tests were considered positive for virus.

Subjects	No. tested	Positive results	
		No.	Percent
Patients with diagnosis of			
AIDS with Kaposi's sarcoma	41	22	53.6
AIDS with opportunistic infection	4	0	0
Lymphadenopathy syndrome	10	5	50.0
Other individuals			
Male sexual partners of AIDS patients*	14	3	21.4
Clinically healthy homosexual men†	9	2	22.2
Clinically healthy heterosexual individuals†	23	1	4.0

*Clinically healthy individuals who had steady sexual contact with a patient for at least 6 months before the patient became ill. †Some of these individuals volunteered for the study.