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Structural similarities between the mammalian β-adrenergic and reovirus type 3 receptors

(anti-idiotype/virus receptor/viral tropism)

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ABSTRACT **The mechanism by which viruses bind to and infect specific tissues to cause disease has only recently begun to be understood. The mammalian reoviruses provide an especially attractive model for studying the details of cell surface recognition. The cell and tissue tropism of reovirus is determined by a portion of the viral hemagglutinin termed the neutralization domain. We have reported previously on the generation of both monoclonal and polyclonal anti-idiotypic antibodies that mimic the viral hemagglutinin in the specificity of binding to the reovirus receptor. By using these anti-idiotypic antibodies as specific probes, we have successfully isolated the mammalian reovirus receptor from neuronal and lymphoid cells. In the present study, we report that the reovirus receptor is structurally similar to the mammalian β-adrenergic receptor. This conclusion is based on the following observations: (*i*) purified β-adrenergic receptor is immunoprecipitable by anti-reovirus receptor antibody; (*ii*) purified reovirus receptor obtained from murine thymoma cells and β-adrenergic receptor obtained from calf lung exhibit identical molecular masses and isoelectric points; (*iii*) trypsin digests of purified reovirus and β-adrenergic receptors display indistinguishable fragment patterns; (*iv*) purified reovirus receptor binds the β-antagonist [^{125}I]iodohydroxybenzylpindolol and this binding is blocked by the β-agonist isoproterenol.**

The susceptibility of cells to viral infection is influenced by events that occur during the initial stages of the interaction (1). This is particularly evident for the mammalian viruses, where infection and subsequent virus disease are largely determined by the ability of viruses to bind to distinct types of cells. Even within a given species, the expression of different genes in different cell types or within the same cell type at different stages of development can result in the formation of different sets of specific virus receptors. The identification of virus receptors and the study of their interactions with ligands are therefore of critical importance in understanding viral tropism and subsequent disease patterns (2, 3).

Over the last few years, there have been a number of studies attempting to identify the nature of the cellular membrane proteins that several viruses have used as receptors. For example, it has been speculated that Semliki Forest virus binds to histocompatibility antigens in humans and mice (4). However, although binding studies have supported this association, Semliki Forest virus can grow in cells devoid of histocompatibility antigens, raising questions as to the significance of the role of histocompatibility antigens as receptors (5). Lactate dehydrogenase virus has been found to interact with mouse Ia antigens, suggesting a role of Ia in targeting virus to a subset of macrophages (6). Rabies virus has been reported to bind in close association with the acetylcholine receptors (7) and Epstein–Barr virus has been shown to recognize the complement receptor type 2 of human lymphocytes (8–10).

Work in our laboratory has centered on the utilization of anti-idiotypic antibodies in the study of the reovirus type 3 cellular receptor (11, 12). Syngeneic monoclonal and xenogeneic polyclonal anti-idiotypic antibodies with specificity for the reovirus receptor have been constructed (13, 14). Reovirus and the anti-idiotypic antibody were observed to have similar biological effects in limiting concanavalin A-induced stimulation of lymphocytes (15) and also can inhibit DNA synthesis in a number of cell lines (unpublished results). These anti-receptor antibodies also specifically inhibited reovirus binding to target tissues in a serotype-specific manner (16).

More recently, we have utilized immunoblotting techniques to show that reovirus type 3 and anti-receptor antibody bind to a 67-kDa cell-surface structure obtained from lymphoid and neuronal cell membranes (17). The receptor molecule has been isolated by immunoprecipitation with rabbit polyclonal anti-receptor antibody. The purified receptor is a monomeric glycoprotein with a molecular mass of 67 kDa, as determined by $NaDodSO_4$/PAGE, and demonstrates a pI of 5.8–6.0.

We observed that tissues that bind mammalian reovirus type 3 express β-adrenergic receptors. These tissues include brain, heart, muscle, and lymphocytes. Since the reported molecular mass and isoelectric point of the β-adrenergic receptor are comparable to that of the isolated reovirus binding protein, we conducted studies using anti-idiotypic antibodies to further examine receptor similarities. In this report, we show that the mammalian reovirus receptor shares extensive structural homology with the mammalian β-adrenergic receptor.

MATERIALS AND METHODS

Anti-Idiotypic Antibodies. The generation of rabbit anti-idiotypic antibodies has been reported (13). Briefly, BALB/c mice were inoculated repeatedly with 10^9 particles of purified reovirus type 3 virions and serum obtained was twice precipitated with 40% saturated ammonium sulfate followed by Sephacryl-200 gel filtration. To enrich for anti-hemagglutinin (anti-HA) antibody, serum was absorbed on type 1 virus and the 3HA1 recombinant reovirus that differs only in the gene encoding the σ 1 protein. The enriched antibodies

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Abbreviations: HA, hemagglutinin; ISO, isoproterenol; IHYP, iodohydroxybenzylpindolol.

(500 μg) in complete Freund's adjuvant were injected intramuscularly and into the footpads of outbred New Zealand rabbits. Rabbits were immunized at 3 and 5 weeks with the same emulsion, and were bled 7 days after the last injection. Serum was purified by precipitation twice in 40% saturated ammonium sulfate and then absorbed with normal mouse serum. The binding specificity of this enriched antiserum was demonstrated by affinity for HA3-specific immunoglobulin by using solid-phase radioimmunoassay. The rabbit anti-idiotypic antibodies were also shown to bind the anti-HA3-specific monoclonal antibody 9BG5 and to block the binding of antibody 9BG5 to ^{125}I-labeled HA3.

Isolation of Reovirus Receptor. Reovirus receptor was purified from murine thymoma cells R1.1 (17). Cells (2×10^7) were surface-labeled with ^{125}I by using enzymobead lactoperoxidase (Bio-Rad). Briefly, cells were mixed with 1 mCi (1 Ci = 37 GBq) of Na^{125}I (New England Nuclear) in 50 μl of 0.2 M sodium phosphate (pH 7.2) and 25 μl of 1% β-D-glucose; then 50 μl of hydrated enzymobead reagent was added and the reaction mixture was incubated at room temperature for 30 min. The reaction was stopped by extensive washing in phosphate-buffered saline. Membrane proteins were solubilized following incubation in phosphate-buffered saline containing 0.5% Triton X-100, 0.25% Nonidet P-40, and 1 mM phenylmethylsulfonyl fluoride for 30 min on ice. The debris was cleared by centrifugation at 30,000 × g for 60 min. Aliquots containing 300,000 cpm were incubated with 25 μg of anti-receptor antibody for 60 min at room temperature. Twenty-five microliters of Sepharose-protein A (Pharmacia) was next added and the incubation was continued for 2 hr at room temperature. Beads were collected by centrifugation in a Beckman Microfuge, washed three times in solubilization buffer, and then washed twice in phosphate-buffered saline. Precipitated receptors were identified by NaDodSO$_4$/PAGE and two-dimensional gel electrophoresis.

Purification of β-Adrenergic Receptor. β-adrenergic receptor was purified from calf lung (18, 19). Briefly, membranes were prepared and solubilized with 0.4% digitonin/0.08% cholic acid in 0.1 M Tris·HCl, pH 7.5/5 mM EDTA/1 mM $MgCl_2$. The solubilized fraction was passed through an acebutolol affinity column and eluted with alprenolol. The eluent was further purified with HPLC/gel-exclusion chromatography and another cycle of affinity chromatography. The eluent was collected and iodinated with ^{125}I. The labeled protein was dialyzed extensively to remove the free isotope and stored at −20°C until used.

Two-Dimensional Gel Electrophoresis. Immunoprecipitated proteins were adjusted to isoelectric focusing sample buffer (9.5 M urea/2% Nonidet P-40/1.6% Pharmalyte, pH 5–8/0.4% Pharmalyte, pH 3–10/5% 2-mercaptoethanol) (20); Pharmalyte was from Pharmacia. The sample (50 μl) was loaded into the basic end of 120 × 2.8 mm (diameter) tube gels containing 9.2 M urea, 4% acrylamide, 2% Nonidet P-40, 1.6% Pharmalyte (pH 5–8), and 0.4% Pharmalyte (pH 3–10) and run at 500 V for 16 hr. The tube gels were then incubated in the Laemmli sample buffer (21) for 1 hr and layered on top of a 10% NaDodSO$_4$/PAGE slab gel run at 30 mA for 5 hr. The gels were fixed, dried, and exposed to a Kodak X-Omat AR film for 2 days at −70°C.

Trypsin Digestion. Receptors purified as described above were incubated with 50 μl of L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin (Sigma) (500 μg/ml) in 10 mM ammonium bicarbonate at 37°C for 16 hr. An additional 10 μl of trypsin solution was added and incubation was continued for 2 hr. The solution was adjusted to Laemmli sample buffer and run on a 10% NaDodSO$_4$/PAGE slab gel.

Iodohydroxybenzylpindolol (IHYP) Binding. [^{125}I]IHYP was obtained from New England Nuclear. Twenty thousand cpm of IHYP was incubated with 1×10^6 R1.1 cells, with or without 1 mM isoproterenol (ISO), for 30 min at 37°C. Then cells were washed and the membranes were solubilized with 0.5% digitonin. Debris were cleared by centrifugation. The reovirus receptor was precipitated by the addition of anti-idiotypic antibodies and was recovered by Sepharose-protein A. In the control experiments, anti-idiotypic antibodies were replaced by normal rabbit antibodies. After washing, pellets were assayed for radioactivity in a Beckman γ counter. Binding of IHYP to isolated receptor was performed by incubating the receptor protein isolated as described above, with or without 1 mM ISO, with 20,000 cpm of IHYP for 1 hr at 37°C, followed by several washings with Tris/saline buffer (10 mM Tris·HCl/150 mM NaCl, pH 7.4). The mean ± SD was calculated from triplicate measurements.

RESULTS

Comparison of Reovirus and β-Adrenergic Receptors. Based on the tissue distribution and biochemical characterization of reovirus type 3 and β-adrenergic receptors, we conducted preliminary studies using anti-idiotypic antibodies to more fully examine the similarities of these receptors. Initial results indicated that affinity-purified ^{125}I-labeled β-adrenergic receptor protein could be bound specifically by anti-reovirus receptor antibody but not by normal immunoglobulins (Table 1). These data demonstrate that reovirus and the β-adrenergic receptors share an epitope(s) recognized by the anti-reovirus-receptor antibody.

To determine the extent of homology between reovirus and β-adrenergic receptors, more detailed biochemical studies were conducted by using two-dimensional gel electrophoresis and enzyme digestion. Fig. 1 shows the autoradiograms of immunoprecipitates from R1.1 extracts (Fig. 1*A*) and affinity-purified β-adrenergic receptor (Fig. 1*B*) analyzed by two-dimensional gel electrophoresis. The two maps are strikingly similar. In agreement with our previous observation, we detected a structure with a molecular mass of 67 kDa and a pI of 5.8–6.0. The similarity of the patterns indicates that the two receptors not only share antigenic epitopes but are structurally similar.

To further compare the reovirus binding structure and the β-adrenergic receptor, we examined the trypsin digestion patterns of the reovirus receptor isolated from R1.1 thymoma line and the β-adrenergic receptor purified from calf lung. An autoradiogram of the trypic maps for the two proteins is shown in Fig. 2. Indistinguishable digestion fragments were observed for both proteins, with a major fragment of 50 kDa and several minor fragments of 57 and 25 kDa. This further demonstrates that the two receptors are structurally essentially identical.

Binding of IHYP to Reovirus Receptor. We next evaluated the capacity of reovirus receptors to bind the β-antagonist [^{125}I]IHYP. This was accomplished by measuring the amount of membrane-bound IHYP that coprecipitates with the reovirus receptor and the binding of IHYP to purified reovirus receptor. In both instances the specificity of this interaction was verified by inhibition of binding with the

Table 1. Binding of ^{125}I-labeled reovirus and β-adrenergic receptors to anti-idiotypic antibodies

Receptor	Anti-idiotype, cpm	Normal Ig, cpm
Reovirus	3530	121
β-Adrenergic	3018	250

Lysate from surface-labeled R1.1 cells and labeled affinity-purified β-adrenergic receptor from calf lung were each incubated with 10 μg of anti-idiotypic antibodies or normal immunoglobulins for 2 hr at room temperature. The immune complexes were recovered subsequently by Sepharose-protein A and washed extensively. The beads were then assayed for radioactivity in a γ counter.

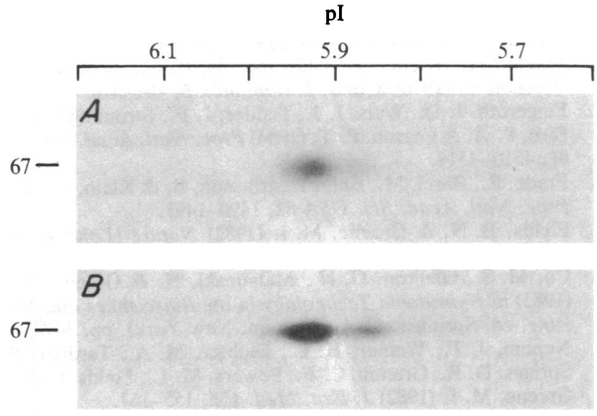

FIG. 1. Two-dimensional gel electrophoresis of immunoprecipitated reovirus type 3 receptor and β-adrenergic receptor. (*A*) Reovirus receptor isolated from murine thymoma R1.1 cells. (*B*) β-Adrenergic receptor affinity-purified from calf lung. Both receptors show a molecular mass of 67 kDa and a pI of 5.8–6.0.

unlabeled β-agonist ISO. The open bars shown in Fig. 3 demonstrate the level of IHYP binding to R1.1 cell surface. The level of binding was reduced by 90% when reactions were conducted in the presence of 1 mM ISO. Similar results were obtained with isolated receptors. The level of IHYP binding to isolated receptor proteins was approximately twice the level of nonspecific binding. This is most likely a consequence of the solubilization procedure utilized, which often causes receptors to partially lose their ligand-binding conformation, as has been shown by others. This level of

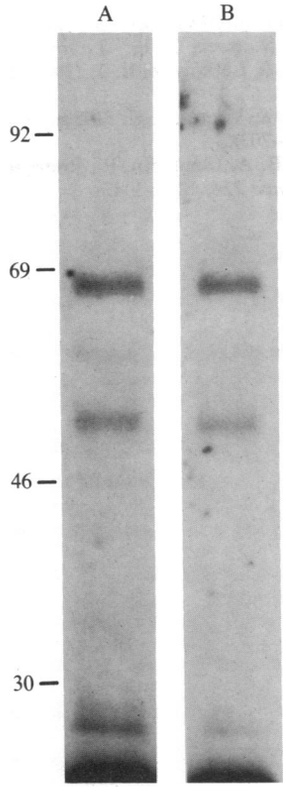

FIG. 2. Partial trypsin digestion of reovirus receptor (lane A) and β-adrenergic receptor (lane B). In addition to the undigested bands of 67 kDa, both proteins show a major fragment of 50 kDa and several minor fragments of 57 and 25 kDa. Molecular mass standards are shown in kDa.

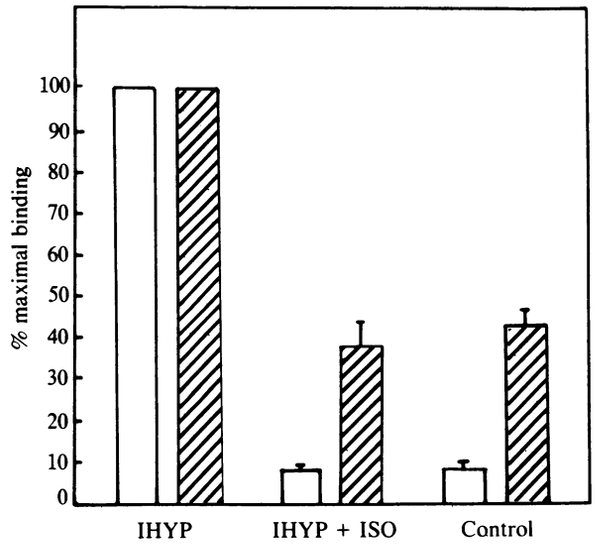

FIG. 3. Binding of IHYP to immunoprecipitated reovirus receptors. The extent of IHYP binding, and competition by the β-agonist ISO, to the reovirus receptor was assessed by measuring the amount of cell-bound IHYP that was coprecipitated by anti-idiotype (open bars) and the binding of IHYP to anti-idiotype-purified receptor (hatched bars). In both instances precipitations with anti-idiotype were conducted with IHYP (20,000 cpm, 2200 Ci/mmol) or IHYP + ISO (1 mM) for 30 min at 37°C. In the control experiments, anti-idiotypic antibodies were replaced by normal rabbit immunoglobulins. Results are expressed as a percentage of maximal IHYP binding and correspond to 1897 ± 42 cpm for coprecipitations and 2560 ± 616 cpm for binding to purified receptors. The mean ± SD was calculated from triplicate measurements.

binding shows that the reovirus receptor may indeed contain a β-ligand binding site.

DISCUSSION

Pharmacological approaches have delineated two types of β-adrenergic receptors (22). β_1- and β_2-adrenergic receptors stimulate the membrane-bound enzyme complex adenylate cyclase, which catalyzes the formation of cyclic AMP from the substrate ATP. Radioligand binding techniques have been used to determine the tissue distribution of β-adrenergic receptor subtypes. Though most tissues have been shown to contain both β_1 and β_2 receptors, mammalian hearts and brains are particularly rich in β_1 receptors, whereas lungs have high levels of β_2 receptors. Purification of β-adrenergic receptors from several sources has been accomplished by using ligand affinity chromatography (23). The data available thus far suggest that both mammalian β_1- and β_2-adrenergic receptors are polypeptides with molecular masses in the range of 52–70 kDa (24, 25). This diversity may possibly reflect the existence of isoreceptors differing, for example, in their extent of glycosylation or they may more likely represent the products of limited proteolysis during the preparation of samples for analysis (18, 19). It appears that all mammalian β receptors thus far characterized contain a principal binding structure with size of 62–67 kDa.

The purified β-adrenergic receptor used here for comparison to the reovirus receptor was obtained from calf lung tissue thought to be predominantly composed of the β_2 subtype. However, the tissues known to be susceptible to reovirus infection include the brain, heart, and skeletal muscles (unpublished work). These tissues contain predominantly either β_1 or β_2 receptors. One possibility to explain the ability of the antibody to bind both receptors is that the anti-receptor antibodies recognize a common domain shared by both β_1 and β_2 subtypes. It has been reported that a

monoclonal antibody, FV-104, directed to a region within the ligand binding site of the β receptors crossreacts equally with β_1 and β_2 receptors (26). Since both receptor subtypes bind the same ligands and are distinguished only by their relative binding affinities, a likely possibility is that the two receptor subtypes are structurally similar with only minor amino acid and/or carbohydrate residue differences. The two-dimensional maps of the β-adrenergic and reovirus receptors that suggest heterogeneity of the 67-kDa structure are consistent with this interpretation.

A variety of viruses has been thought to interact with cell-surface components (4–9). Whether these viruses utilize these same cell-surface proteins to gain entry into cells is not clear. We have shown recently that the same anti-receptor antibodies we have described can prevent reovirus binding and infection of susceptible tissues (unpublished results). The demonstration that reovirus type 3 binds to β-adrenergic receptors is consistent with the idea that physiologic receptors may be utilized by pathogens for entry into tissues. Viral interactions with physiologic receptors may also provide an explanation of metabolic disorders caused by noncytopathic viruses or autoantibodies. The etiology of non-insulin-dependent diabetes mellitus may illustrate such an effect of lymphocytic choriomeningitis virus (27).

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1. Dimmock, N. J. (1982) *J. Gen. Virol.* **59,** 1–22.
2. Meager, A. & Hughes, R. C. (1977) in *Receptors and Recognition*, eds. Cuatrecases, P. & Greaves, M. F. (Chapman & Hall, London), Ser. A, No. 4, pp. 143–195.
3. Tardieu, M., Epstein, R. E. & Weiner, H. L. (1983) *Int. Rev. Cytol.* **80,** 27–61.
4. Helenius, A., Morein, B., Fries, E., Simons, K., Robinson, P., Schirrmacher, V., Terhorst, C. & Strominger, J. L. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3846–3850.
5. Oldstone, M. B. A., Tishon, A., Dutko, F. J., Kennedy, S. I. T., Holland, J. J. & Lampert, P. W. (1980) *J. Virol.* **34** 256–265.
6. Inada, T. & Mims, C. A. (1984) *Nature (London)* **309,** 59–61.
7. Lentz, T. L., Burrage, T. G., Smith, A. L., Crick, J. & Tignor, G. H. (1982) *Science* **215,** 182–184.
8. Jondal, M., Klein, G., Oldstone, M. B. A., Bokish, V. & Yefenof, E. (1976) *Scand. J. Immunol.* **5,** 401–410.
9. Fingeroth, J. D., Weis, J. J., Tedder, J. F., Strominger, J. L., Biro, P. A. & Fearon, P. T. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4510–4514.
10. Frade, R., Barel, M., Ehlin-Henriksson, B. & Klein, G. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1490–1493.
11. Fields, B. N. & Greene, M. I. (1982) *Nature (London)* **300,** 19–23.
12. Co, M. S., Gaulton, G. N., Matsuzaki, N. & Greene, M. I. (1985) in *Hybridoma Technology in the Biosciences and Medicine*, ed. Springer, T. A. (Plenum, New York), pp. 341–351.
13. Nepom, J. T., Weiner, H. L., Dichter, M. A., Tardieu, M., Spriggs, D. R., Gramm, C. F., Powers, M. L., Fields, B. N. & Greene, M. I. (1982) *J. Exp. Med.* **155,** 155–167.
14. Noseworthy, J. H., Fields, B. N., Dichter, M. A., Sobotka, C., Pizer, E., Perry, L. L., Nepom, J. T. & Greene, M. I. (1983) *J. Immunol.* **131,** 2533–2538.
15. Nepom, J. T., Tardieu, M., Epstein, R. L., Noseworthy, J. H., Weiner, H. L., Gentsch, J., Fields, B. N. & Greene, M. I. (1982) *Surv. Immunol. Res.* **1,** 255–261.
16. Kauffman, R. S., Noseworthy, J. H., Nepom, J. T., Finberg, R., Fields, B. N. & Greene, M. I. (1983) *J. Immunol.* **131,** 2539–2541.
17. Co, M. S., Gaulton, G. N., Fields, B. N. & Greene, M. I. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1494–1498.
18. Homcy, C. J., Rockson, S. G., Countaway, J. & Egan, D. (1983) *Biochemistry* **22,** 660–668.
19. Insoft, R. & Homcy, C. J. (1984) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **43,** 158A (abstr.).
20. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
21. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
22. Lands, A. M., Arnold, A., McAuliff, J. P., Ludwena, F. P. & Brown, T. G., Jr. (1967) *Nature (London)* **214,** 587–588.
23. Lefkowitz, R. J., Stadel, J. M. & Caron, M. G. (1983) *Annu. Rev. Biochem.* **52,** 159–185.
24. Benovic, J. L., Stiles, G. L., Lefkowitz, R. J. & Caron, M. G. (1983) *Biochem. Biophys. Res. Commun.* **110,** 504–511.
25. Stiles, G. I., Strasser, R. H., Lavin, T. N., Jones, L. R., Caron, M. G. & Lefkowitz, R. J. (1983) *J. Biol. Chem.* **258,** 8443–8449.
26. Fraser, C. M. & Venter, J. C. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 7034–7038.
27. Oldstone, M. B. A., Southern, P., Rodriguez, M. & Lampert, P. (1984) *Science* **224,** 1440–1442.