Zaprinast (100 fLM; May & Baker) was added to all samples. Zaprinast itself prevented cGMP hydrolysis by PDE during the experiment the PDE inhibitor for several other classes of GTP-binding proteins (for example increases GTPase rates by at least twofold, close to the turn-off factor). The maximal GTPase rate observed in this reconstitution was added at a saturating concentration of 100 fLM where indicated. To prevent cGMP hydrolysis by PDE during the experiment the PDE inhibitor Zaprinast (100 fLM; May & Baker) was added to all samples. Zaprinast itself did not influence the GTPase reaction (not shown). All the data are taken from one of three similar experiments and approximated by single exponents for the membranes in the presence of both PDE and cGMP and 0.13 s⁻¹ for all other conditions.

fast termination of PDE activation observed under similar conditions. More recent work indicates that transducin GTPase can be faster under more physiological conditions, but the mechanism of GTPase activation has remained unclear. The data of Fig. 1 show that PDE itself serves as a GTPase-activating factor. The maximal GTPase rate observed in this reconstitution study (~0.15 s⁻¹) is still about 10-fold slower than the rate of the recovery from a photoresponse. But a more rapid rate (~0.6 s⁻¹) is observed in suspensions of disrupted rod outer segments for the fast component of GTPase suppressed by micromolar concentrations of cGMP. Our study allows us to conclude that this faster GTPase is a property of the transducin GTPase-dependent G protein activation is higher in rod outer segment suspensions than in reconstituted membranes. More recent data (V.Y.A. et al., manuscript in preparation) shows that further concentration of rod outer segment suspensions (≥100 μM rhodopsin) increases GTPase rates by at least twofold, close to the turn-off time of the photoresponse.

The data shown in Fig. 2 indicate a feedback mechanism in retinal rods based on cGMP-dependent regulation of the lifetime of activated PDE. Such a mechanism might function during rod background adaptation, when the duration and light sensitivity of the photoresponse is diminished. A reasonable model is that background light depletes intracellular cGMP levels, causing dissociation of cGMP from the non-catalytic binding sites on PDE. This would accelerate the GTPase activity that terminates each PDE activation event, leading to a faster and/or smaller photoresponse. Such a mechanism might work in parallel with the known calcium feedback regulation of adaptation. The regulation of GTP-binding protein GTPase activity by an effector described here, although not previously described for a heterotrimeric G protein, has been documented extensively for several other classes of GTP-binding proteins (for example ref. 26) It is observed for elongation and initiation factors whose GTPase activity is enhanced by ribosomes. The class of small GTP-binding proteins including the product of proto-oncogene ras interact with GTPase-activating proteins (GAPs) that may also be their effectors. The intrinsic GTPase of the heterotrimeric signal-transducing G proteins is considerably more rapid than that of the small GTP-binding proteins (for example ref. 25) but still in several systems such as photoreception and olfaction, and muscarinic receptor-induced potassium channel regulation it has seemed to be too slow to explain the rapid on-off cycle of the relevant effectors. Because acceleration has now been associated with the effector enzyme in the photoreceptor, it is relevant to search for similar mechanisms in other systems using heterotrimeric G proteins.

FIG. 2. cGMP reverses transducin GTPase acceleration by PDE but not PDE. Single-turnover GTPase measurements were done as described in the legend to Fig. 1. Subsaturating concentrations of PDE (0.2 fLM) and PDE (0.4 fLM) which caused the same extent of GTPase acceleration were used. GTPase was added at a saturating concentration of 100 μM where indicated. To prevent cGMP hydrolysis by PDE during the experiment the PDE inhibitor Zaprinast (100 μM; May & Baker) was added to all samples. Zaprinast itself did not influence the GTPase reaction (not shown). All the data are taken from one of three similar experiments and approximated by single exponents revealing GTPase rates of 0.031 s⁻¹ for test membranes alone. 0.053 s⁻¹ for the membranes in the presence of both PDE and cGMP and 0.13 s⁻¹ for all other conditions.

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Aminopeptidase N is a major receptor for the enteropathogenic coronavirus TGEV

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CORONAVIRUSES, like many animal viruses, are characterized by a restricted host range and tissue tropism. Transmissible gastroenteritis virus (TGEV), a major pathogen causing a fatal diarrhoea in newborn pig, replicates selectively in the differentiated enterocytes covering the villi of the small intestine. To investigate the molecular determinants of the infection, we characterized the scrapie-like virus used by the virus for binding and entry into host cells. Here we report that aminopeptidase N, an ectoenzyme abundantly expressed at the apical membrane of the enterocytes, serves as a receptor for TGEV. Monoclonal antibodies were selected for their ability to block infection by TGEV of porcine cell lines. They recognized a brush-border membrane protein of Mr 150,000, which was identified as aminopeptidase N by amino-terminal sequencing. Aminopeptidase N is an endopeptidase, which is a membrane protein with a molecular weight of 150,000 and is recognized by monoclonal antibodies. The aminopeptidase N is a receptor for the entero...
METHODS. Anchor-free APN was purified by immunoadsorbent Chromatogra­
either G43 or a control antibody and tested for TGEV binding as above.

were purified as described

coated with TGEV, BCV or rotavirus virions. Bound APN was revealed by
western blotting. a. APN (0.2 μg) was added with a 50 μg virion suspension in cell
culture medium. After 1 h at 37 °C, the virions were pelleted through a 10%
glycerol cushion by centrifugation at 150,000g for 30 min. One TGEV sample
was mixed with APN preincubated with G43 IgGs (200 μg ml⁻¹). APN bound
to virus was revealed by western blotting using rabbit IgGs directed against
denatured APN19 and a peroxidase conjugate. b. APN (0.6 μg) was added to
virus-coated wells (1 μg per well) for 1 h at 37 °C after incubation (or mock
incubation) with dilutions of G43 IgGs at 200 μg ml⁻¹. After washes with PBS plus
0.05% Tween 20, bound APN was detected by rabbit IgGs against native APN and a phosphatase conjugate.
Further evidence that the anti-TGEV-receptor antibodies recognized aminopeptidase N was obtained by showing that (1) an antibody raised against rabbit aminopeptidase N reacted with the same three polypeptides in brush-border membrane preparations (Fig. 2, lane 2): 95K and 50K, corresponding to the B (amino) and C (carboxy) subunits of the pig aminopeptidase5,12; (2) the immunoprecipitated material hydrolysed leucine p-nitroanilide, a chromogenic substrate specific for aminopeptidase (ref. 6; data not shown).

Two experiments were designed to demonstrate any direct association between aminopeptidase N and the virus. First, soluble aminopeptidase N was centrifuged after incubation in the presence of virions (Fig. 3b), it bound to TGEV and not the other enteric viruses. In both assays, earlier incubation with an antibody against aminopeptidase N reduced the binding considerably. Because the two components were purified to homogeneity, it was concluded that the interaction between the aminopeptidase and TGEV occurs in the absence of any other cellular protein.

The gene encoding aminopeptidase N (APN) was expressed in non-permissive cells to see whether this would confer them with the capacity to bind TGEV. A pig intestine complementary DNA library was screened by use of a homologous DNA probe derived from the 5' end of APN gene. A full-length cDNA copy was cloned and contained an open reading frame of 2,889 nucleotides encoding a polypeptide 79% identical to human aminopeptidase N (data not shown). MDCK cell clones stably transformed with the porcine APN cDNA expressed a polypeptide of 150K which reacted with antibodies against aminopeptidase N (Fig. 4a). The aminopeptidase activity of the transfected clones was about 40-fold higher compared with non-transformed clones. On viral challenge, all of the three independent clones tested seemed to be fairly susceptible to TGEV infection, as proved by extensive destruction of the infected monolayers and synthesis of the viral structural polypeptides (Fig. 4b, c). Earlier incubation with an antibody specific for aminopeptidase N prevented the appearance of viral cytopathic effect. These results show that aminopeptidase N was the only porcine protein necessary to confer susceptibility on canine kidney cells naturally resistant to TGEV. Moreover, the protease function of the molecule did not seem to be involved because it was blocked by bestatin, an inhibitor of aminopeptidase, without preventing the infection (Fig. 4b).

So far, defined receptors include molecules that belong to the immunoglobulin superfamily, such as CD4 for HIV, ICAM-1 for rhinovirus, poliovirus receptor and a carcinoembryonic antigen for murine hepatitis coronavirus and also an amino-acid transporter for murine leukaemia retroviruses. Our study provides strong evidence that porcine aminopeptidase N serves as a receptor for an enveloped RNA virus, TGEV. This emphasizes the diversity of the membrane-bound proteins that viruses subvert for gaining entry into cells.

Aminopeptidase N is a well documented ectoenzyme that binds to the membrane through an N-terminal segment. Human aminopeptidase N is identical to CD13, a surface antigen of many myeloid cells. It is a zinc-binding protease that catalyses the removal of N-terminal, preferentially neutral residues from peptides. It is expressed in many tissues at different levels, the highest activity being found in the small intestinal mucosa, where the aminopeptidase represents about 8% of the protein content of the apical membrane of the differentiated enterocytes, and in the kidney brush border. It is also expressed to a lesser extent in liver, lung and colon, where the virus does replicate, but without causing the specific histopathological damage seen in the small intestine. In the intestine, the distribution of the receptor and the site of multiplication of TGEV are thus strikingly correlated. This argues for a pivotal role of aminopeptidase N/CD13 in determining the tissue tropism of TGEV. Investigating the nature of the virus interaction with aminopeptidase N could provide a rationale for the design of an antiviral strategy against TGEV and related infections.

Fig. 3 legend. Colorimetric assays were done 16h after infection at a multiplicity of five plaque-forming units. Monolayers were fixed and stained with a crystal violet solution. The dye incorporated in cells surviving the viral CPE was measured by optical absorbance after solubilization in acetic acid. Cells were incubated in the presence of G43 antibody or bestatin (1 mM) from 1h before infection. Immunoprecipitation of 35S-methionine-labelled intracellular polypeptides was as described for Fig. 2.
Human aminopeptidase N is a receptor for human coronavirus 229E

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HUMAN coronaviruses (HCV) in two serogroups represented by HCV-229E and HCV-OC43 are an important cause of upper respiratory tract infections. Here we report that human aminopeptidase N, a cell-surface metalloprotease on intestinal, lung and kidney epithelial cells, is a receptor for human coronavirus strain HCV-229E, but not for HCV-OC43. A monoclonal antibody, RBS, blocked HCV-229E virus infection of human lung fibroblasts, immunoprecipitated aminopeptidase N and inhibited its enzymatic activity. HCV-229E-resistant murine fibroblasts became susceptible to HCV-229E replication with complementary DNA encoding human aminopeptidase N. By contrast, infection of human cells with HCV-OC43 was not inhibited by antibody RBS and expression of aminopeptidase N did not enhance HCV-OC43 replication in mouse cells. A mutant aminopeptidase lacking the catalytic site of the enzyme did not bind HCV-229E or RBS and did not render murine cells susceptible to HCV-229E infection, suggesting that the virus-binding site may lie at or near the active site of the human aminopeptidase molecule.

To develop a monoclonal antibody against the HCV-229E receptor, we produced hybridomas against deoxycholate-solubilized membrane proteins of two HCV-229E-susceptible human cell lines (WI38 lung fibroblasts and HL60 myeloid leukaemia cells). A monoclonal antibody designated RBS protected WI38 and RD human cell lines from HCV-229E-induced cytopathic effects and protected WI38 cells from virus infection (Fig. 1a-c). RBS pretreatment reduced the number of HCV-229E-infected WI38 cells at 10 h post-infection by 96%, compared with cells pretreated with control mouse ascites. By contrast, RBS did not inhibit replication of HCV-OC43 in WI38 or RD cells, indicating that the receptor specificities of HCV-OC43 and HCV-229E are different.

Susceptibility to HCV-229E infection in mouse–human somatic cell hybrids depends on a gene located on human chromosome 15 (ref. 6). A promising candidate for the HCV-229E receptor is human aminopeptidase N (hAPN; EC 3.4.11.2), a cell-surface glycoprotein encoded by a gene on bands q23–q26 of human chromosome 15 (ref. 7) and expressed on human lung, renal and intestinal epithelial cells, fibroblasts and nerve synapses. This exopeptidase removes amino-terminal residues to complete the digestion of short peptides in the gut and helps break down neurotransmitter peptides in the brain. hAPN is identical to CD13, a glycoprotein identified on granulocytes, monocytes and their bone marrow progenitors. Porcine aminopeptidase N is a receptor for transmissible gastroenteritis virus, a porcine coronavirus in the same serogroup as HCV-229E (ref. 11). Because aminopeptidase N from humans, pigs and other mammals are structurally similar, we investigated whether HCV-229E and RBS would bind specifically to hAPN and whether expression of hAPN by murine cells would make them susceptible to infection with HCV-229E.

Murine NIH3T3 cells transfected with hAPN cDNA in a retroviral vector (hAPN-3T3) and untransfected NIH3T3 cells were challenged with HCV-229E and HCV-OC43 to determine their susceptibility to virus infection. Although the control NIH3T3 cells were resistant to HCV-229E infection (Fig. 1d), the hAPN-transfected mouse cells were susceptible to infection with this virus (Fig. 1e). By contrast, hAPN-3T3 cells were no more susceptible than NIH3T3 cells to infection with HCV-OC43 (data not shown). Thus, expression of hAPN confers HCV-229E susceptibility, but not HCV-OC43 susceptibility, on murine cells.

We analysed binding of RBS to membrane preparations from hAPN-3T3 or parental NIH3T3 fibroblasts. The antibody bound to membranes of hAPN-3T3 but not to those of NIH3T3 cells (Fig. 2a), indicating that RBS recognized hAPN. Similarly, HCV-229E virions bound more strongly to hAPN-3T3 membranes than to NIH3T3 membranes (Fig. 2b), and RBS competitively inhibited binding of hAPN-3T3 membranes to HCV-229E (Fig. 2c). Binding of antibodies to hAPNmut-3T3 cells was measured by flow cytometry, as outlined in the legend to Fig. 3. The mutant lacks peptidase activity, thus, assays for chemical inhibition were not applicable (NA).

† Binding of antibodies to hAPNmut-3T3 cells was measured by flow cytometry, as outlined in the legend to Fig. 3. The mutant lacks peptidase activity; thus, assays for chemical inhibition were not applicable (NA).
‡ Confluent monolayers of WI38 cells in 96-well plates were pretreated with dilutions of antibodies or inhibitors in medium for 1 h, and then challenged with 1 x 10⁵ p.f.u. per well of HCV-229E. After 1 h of adsorption, the inoculum was removed, and the cells were incubated with fresh medium containing antibodies or inhibitors for 48 h, at which time the monolayers were examined for virus-induced cytopathic effects. Such effects were evident in HCV-229E-infected controls pretreated with normal serum, but not in mock-infected controls. Plus signs, HCV-229E-induced cytopathic effects were inhibited by antibodies up to a dilution of 1:200. All incubations were at 37°C.
§ Inhibitors were tested at the following concentrations: bestatin, 1 mg ml⁻¹; 1,10-phenanthroline, 1.5 mM; 2,2'-dipyridyl, 2.5 mM; actinonin, 2.7 mM. Antibodies were tested at concentrations that saturated available binding sites in flow cytometric assays.

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| Monoclonal antibodies | Inhibition of enzyme activity (%) | Binding to hAPNmut-3T3† | Inhibition of HCV-229E infection‡ |
|-----------------------|---------------------------------|------------------------|-------------------------------|
| WM15                  | 91                              | NA                     | +                             |
| RBS                   | 90                              | NA                     | +                             |
| MY7                   | 42                              | NA                     | +                             |
| Chemical inhibitors§  |                                 |                        |                               |
| Actinonin             | 100                             | NA                     |                               |
| Bestatin              | 100                             | NA                     |                               |
| 1,10-Phenanthroline   | 100                             | NA                     | +                             |
| 2,2'-Dipyridyl        | 100                             | NA                     | +                             |

* The inhibition of hAPN activity was determined as described in the legend to Fig. 2d.
† Binding of antibodies to hAPNmut-3T3 cells was measured by flow cytometry, as outlined in the legend to Fig. 3. The mutant lacks peptidase activity; thus, assays for chemical inhibition were not applicable (NA).
‡ Confluent monolayers of WI38 cells in 96-well plates were pretreated with dilutions of antibodies or inhibitors in medium for 1 h, and then challenged with 1 x 10⁵ p.f.u. per well of HCV-229E. After 1 h of adsorption, the inoculum was removed, and the cells were incubated with fresh medium containing antibodies or inhibitors for 48 h, at which time the monolayers were examined for virus-induced cytopathic effects. Such effects were evident in HCV-229E-infected controls pretreated with normal serum, but not in mock-infected controls. Plus signs, HCV-229E-induced cytopathic effects were inhibited by antibodies up to a dilution of 1:200. All incubations were at 37°C.