dissociating to the phenolate at the antibody combining site.
8. A. R. Fersht and Y. Requena, *J. Am. Chem. Soc.* **90**, 7079 (1971).
9. K. D. Janda *et al.*, *ibid.*, in press.
10. M. L. Bender and R. J. Thomas, *ibid.* **83**, 4183 (1961).
11. J. K. Kirsch and E. Katchalski, *Biochemistry* **4**, 884 (1965).
12. W. W. Cleland, *Adv. Enzymol. Relat. Areas Mol. Biol.* **45**, 273 (1977).
13. The two schemes are not mutually exclusive, but at present there is no evidence for two apparent ionizations in the pH-rate profiles.
14.

$$k_{cat} = \frac{k_2k_3k_4k_5OH^-}{k_3OH^-[k_4k_5 + k_2(k_4 + k_5)] + k_4k_5(k_2 + k_{-2})}$$

$$k_{cat}/K_M = \frac{k_1k_2k_3OH^-}{k_3OH^-(k_2 + k_{-1}) + k_{-1}k_{-2}}$$

$$K_M = \frac{[k_3OH^-(k_2 + K_{-1}) + k_{-1}k_{-2}]k_4k_5}{k_1[k_3OH^-(k_4k_5 + k_2k_4 + k_2k_5) + k_4k_5(k_2 + k_{-2})]}$$

At low pH the equations reduce to:

$$k_{cat}(pH < 9.0) \approx \frac{k_2k_3OH^-}{k_2 + k_{-2}}$$

$$k_{cat}/K_M(pH < 9.0) \approx \frac{k_1k_2k_3OH^-}{k_{-1}k_{-2}}$$

At high pH the equations reduce to:

$$k_{cat}(pH > 9.0) \approx \frac{k_2k_4k_5}{k_4k_5 + k_2(k_4 + k_5)}$$

$$k_{cat}/K_M(pH < 9.0) \approx \frac{k_1k_2}{k_2 + k_{-1}}$$

15. The large value for k_3 implies that deacylation may occur through a bound OH^- species.
16. The product of k_{cat} (pH < 9.0) times k_{cat}/K_M (pH < 9.0) divided by k_{cat}/K_M (pH < 9.0) equals $k_{-1}k_2/(k_2 + k_{-1})$ and can be used to evaluate k_{-1}. The term k_{cat}/K_M (pH > 9.0) then can be used to obtain k_1. Similar checks on the internal consistency of the numerical solutions are obtained by evaluating K_M at both extremes of pH, yielding a range from 19 to 30 μM. The experimentally determined values of K_M are 15 μM (pH 6.6) and 40 μM (pH 10).
17. The values of K_M and K_i are the same, within experimental error, under a wide range of experimental conditions: K_M = 180 ± 20 μM, ATE buffer, pH 9.0, 37°C; K_i = 260 ± 80 μM, 50 mM Pipes, 5% dioxane v/v, pH 7.0, 25°C; K_i = 190 ± 60 μM, ATC, 2.5% dioxane v/v, pH 9.8, 25°C.
18. Various checks of the kinetic data for internal consistency show, for example, that as required k_{cat} (pH < 9.0)/k_{cat} (pH > 9.0) ≈ k_{cat}/K_M (pH 9.0)/k_{cat}/K_M (pH < 9.0); k_{cat} (pH < 9.0) divided by k_{cat}/K_M (pH < 9.0) ≈ K_M.
19. It can be shown from the pairs of equations for k_{cat} and k_{cat}/K_M that the apparent pK_a values in the k_{cat} and k_{cat}/K_M profiles for the amide **2** must be equal; for the ester **1** the same is true when $k_{-1} \approx k_5$.
20. B. Zerner, R. P. M. Bond, M. L. Bender, *J. Am. Chem. Soc.* **86**, 3674 (1964).
21. T. Inagami, A. Patchornik, S. S. York, *J. Biochem. (Tokyo)* **65**, 809 (1969).
22. W. P. Jencks and M. Gilchrist, *J. Am. Chem. Soc.* **90**, 2622 (1968).
23. M. L. Bender and F. J. Kezdy, *ibid.* **86**, 3704 (1964).
24. M. L. Bender *et al.*, *ibid.*, p. 3714.
25. W. J. Albery and J. R. Knowles, *Biochemistry* **15**, 5631 (1976).
26. B. A. Barshop, R. F. Wren, C. Frieden, *Anal. Biochem.* **130**, 134 (1983).
27. S. R. Stone and J. F. Morrison, *Biochemistry* **23**, 2753 (1984).
28. Supported in part by an NIH grant GM4385801 to K.D.J. C.L.B. was on research leave from The College of Wooster, Wooster, Ohio, during 1989–1990. We thank M. I. Weinhouse, J. A. Ashley, D. A. Mcleod, and D. M. Schloeder for expert technical assistance.

11 June 1990; accepted 10 September 1990

Dissociation of gp120 from HIV-1 Virions Induced by Soluble CD4

JOHN P. MOORE,* JANE A. MCKEATING, ROBIN A. WEISS, QUENTIN J. SATTENTAU

The CD4 antigen is the high affinity cellular receptor for the human immunodeficiency virus type–1 (HIV-1). Binding of recombinant soluble CD4 (sCD4) or the purified V1 domain of sCD4 to the surface glycoprotein gp120 on virions resulted in rapid dissociation of gp120 from its complex with the transmembrane glycoprotein gp41. This may represent the initial stage in virus-cell and cell-cell fusion. Shedding of gp120 from virions induced by sCD4 may also contribute to the mechanism by which these soluble receptor molecules neutralize HIV-1.

IN THE PREDOMINANT ROUTE OF HIV-1 infection, the virus binds to the cell surface via a high affinity interaction between gp120 and the cellular protein CD4 (*1, 2*). The gp120 protein is held on the virion surface by a noncovalent interaction with gp41 (*3*). Thus, the initial complex between virus and cell comprises gp41-gp120-CD4. Subsequently, pH-independent fusion of the cellular and viral membranes takes place, which allows entry of the viral genome into the cytoplasm (*4*). A similar process probably occurs during the fusion of gp120-gp41–expressing infected cells with CD4-expressing uninfected cells. The precise mechanism by which fusion takes place is unknown (*5*), but the fusigenic domain of the virus has been mapped to the hydrophobic NH_2-terminus of gp41 (*6*). By analogy with other enveloped viruses (*5*), this region in HIV-1 is probably not exposed until the viral and cell membranes are in close apposition. This implies the existence of a mechanism for exposure of the fusigenic domain at a step in virus infection subsequent to receptor binding. One such mechanism would be for the binding of CD4 to trigger a conformational change in gp120 that reveals the fusigenic domain. Here, we show that binding of purified recombinant soluble CD4 (sCD4) (*7*) or the purified V1 domain of sCD4 (*8*) to gp120 causes dissociation of the gp120-gp41 complex, which we suggest may be the initial stage in virus-cell fusion and cell-cell fusion.

To analyze the effect of sCD4 on virion-bound gp120, we devised a method (*9*) for the separation of virions from soluble gp120 and soluble core antigen p24 by gel-exclusion chromatography on Sephacryl S-1000 and for the detection of the viral antigens by enzyme-linked immunosorbent assay (ELISA) (*9, 10*) (Fig. 1). Conventional biochemical methods [for example, SDS–polyacrylamide gel electrophoresis and protein immunoblotting] were insufficiently sensitive for quantitation of the small amounts of HIV-1 antigens present in the maximum volume of viral culture supernatant (100 μl) that could be analyzed by chromatography under viral containment conditions. Preliminary experiments established that infectious HIV-1 was quantitatively eluted from the 2-ml column in or near the exclusion volume (0.6 to 1.2 ml), as expected for particles with a diameter of 100 to 150 nm (*11*) and a gel matrix with a particle exclusion limit of 300- to 400-nm diameter. In contrast, recombinant gp120 and p24 diluted in serum-containing culture medium were recovered quantitatively in broad peaks at an elution volume of 1.2 to 2.4 ml (*12*). The precision with which recovered viral antigens can be estimated is limited by the accuracy of ELISA measurements; in a typical experiment the recoveries (mean ± SD) from six separate chromatographic analyses of samples (50 μl) of the HIV-1 strain $HTLV-III_{RF}$ incubated with and without sCD4 were 2.44 ± 0.22 ng of gp120 and 36.5 ± 4.6 ng of p24. Thus, we could cleanly separate virions (10^8 kD) from soluble viral antigens (10^4 to 10^5 kD) without

J. P. Moore, J. A. McKeating, R. A. Weiss, Chester Beatty Laboratories, The Institute of Cancer Research, London SW3 6JB, United Kingdom.
Q. J. Sattentau, Howard Hughes Medical Institute, College of Physicians and Surgeons of Columbia University, New York, NY 10032.

*To whom correspondence should be addressed.

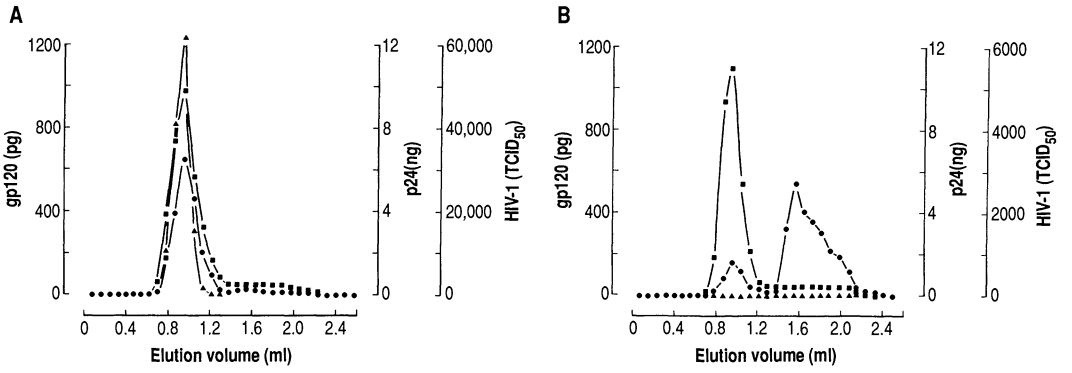

Fig. 1. Chromatography of HIV-1 ($HTLV\text{-}III_{RF}$) on Sephacryl S-1000 after treatment with or without sCD4. Virus (50 μl at a concentration of 10^7 $TCID_{50}$ per milliliter) ($TCID_{50}$ is median tissue culture infectious dose) containing 38 ± 4 ng (mean ± SD) of p24 and 2.4 ± 0.2 ng of gp120 was incubated for 90 min at 37°C without (**A**) or with (**B**) sCD4 (50 μl, final concentration of 50 μg/ml) before gel-exclusion chromatography on Sephacryl S-1000 (*9*). The amount of infectious HIV-1 (▲) in portions of each fraction was determined (*9*), and the remainder of each fraction was inactivated with 1% Empigen detergent before assay of gp120 (●) and p24 (■) by ELISA (*9, 10*). Total recoveries of viral antigens from the columns were (A) 37 ng of p24 and 2.2 ng of gp120 and (B) 36 ng of p24 and 3.0 ng of gp120. The data shown are from paired column elutions and are typical of many such experiments.

systematic loss of either gp120 or p24 (virion or recombinant) during chromatography. We have been unable to adapt our methodology successfully for the detection of gp41 in fractions eluted from the S-1000 columns.

When a high-titer stock of $HTLV\text{-}III_{RF}$ was analyzed by S-1000 column chromatography, 89% of the p24 and 90% of the gp120 present in the culture were recovered in the infectious virus peak (Fig. 1A). However, after treatment of $HTLV\text{-}III_{RF}$ with sCD4 (50 μg/ml) for 90 min at 37°C, 87% of the p24 but only 15% of the gp120 were present in the virus peak and no infectious virus could be detected (Fig. 1B). The gp120 that was lost from the virus was recovered in the soluble antigen fraction (Fig. 1B), where it was present as a complex with sCD4 (*12*). Treatment with sCD4 therefore caused 83% of the gp120 (but none of the p24 core antigen) initially present on the virions to dissociate from gp41 into the medium. Prior incubation of sCD4 with a tenfold excess of the OKT4A monoclonal antibody (MAb) to CD4—which blocks HIV-1 infection (*1*) and gp120-sCD4 binding (*8, 13*)—inhibited gp120 shedding substantially (Table 1). A specific sCD4-gp120 interaction was therefore required for initiation of gp120 dissociation from gp41.

The rate of shedding of gp120 from HIV-1 was biphasic; most dissociated within 10 min of sCD4 addition, but additional gp120 was slowly lost from the virions over a 2-hour period (Fig. 2A). An sCD4 concentration of 0.3 μg/ml (7 nM) was sufficient to cause significant gp120 loss from HIV-1 at 37°C (Fig. 2B), and half-maximal reduction of bound gp120 and inhibition of viral infectivity both occurred at an sCD4 concentration of ~1 μg/ml. Thus, loss of virion gp120 may contribute to the neutralizing action of sCD4, and inhibition by this mechanism is essentially irreversible. Competitive inhibition by sCD4 of the virus-cell interaction (*14, 15*) is not, however, excluded by our data; some gp120 remains on the virus particle even after extensive treatment with sCD4, and we can also detect (by ELISA) sCD4 in the virus peak fractions (*12*).

Detailed measurements of both the binding of sCD4 to virions at 37°C and the consequent loss of gp120-sCD4 complexes indicate that the extent of sCD4 binding at each sCD4 concentration is regulated by the chemical association constant for the interaction between monomeric gp120 and sCD4 molecules (*12*), which is of the order of 1×10^9 M^{-1} (*7, 13, 15*). This supports the conclusion reached by Layne *et al.* (*15*) from mathematical analyses of quantitative infectivity assays. A large proportion (>50%) of the gp120 molecules on the virions must be occupied by sCD4 before there is significant dissociation of gp120 from gp41 (*12*). This may suggest that each of the individual molecules of a gp120 oligomer (*16, 17*) must be bound by sCD4 for dissociation of the entire oligomer from the virion. At 4°C there was no significant loss of gp120 from virions within 2 hours of addition of saturating sCD4 concentrations (50 μg/ml) (*12*). Both this observation and the slowness with which gp120 dissociates from virions at 37°C (Fig. 2A) are probably attributable to a requirement for thermal energy to drive conformational changes involved in breaking the noncovalent association between gp120 and gp41.

Dissociation of gp120 induced by sCD4 was also observed with the $HTLV\text{-}III_B$ and $HTLV\text{-}III_{MN}$ isolates of HIV-1 (Table 1). We have noted, however, that in general, the greater the spontaneous dissociation of gp120 from virions (*18*), the smaller (proportionately) the dissociation induced by sCD4. A minor proportion (10 to 20%) of the gp120 molecules on the HIV-1 strains we have studied may be resistant to spontaneous (slow) dissociation and to sCD4-mediated (rapid) dissociation from gp41. The reason for this gp120 heterogeneity is not known, and we cannot determine with our methodology whether a proportion of the virion population is completely resistant to sCD4 or whether each virion contains resistant gp120 molecules. Analysis by electron microscopy (*19*) suggests, however,

Table 1. Effect of antibodies on sCD4-induced gp120 shedding from HIV-1. Viruses [$HTLV\text{-}III_{RF}$ (RF), $HTLV\text{-}III_B$ (IIIB), and $HTLV\text{-}III_{MN}$ (MN)] were incubated for 90 min at 37°C with the reagents indicated before S-1000 chromatography (*9*). The amounts of gp120 and p24 in the infectious virus and soluble antigen peaks were summed and the percentages of virion-bound gp120 and p24 determined (*9, 10*). Concentrations were as follows: sCD4, 10 μg/ml (100 μg/ml for MN); V1 domain of sCD4 (V1-sCD4) (*8*), 2.5 μg/ml (molar equivalent of sCD4 at 10 μg/ml); MAb OKT4A (*1*), 100 μg/ml; MAb 536 (*22*), 10 μg/ml; MAb Q425, (*23*), 100 μg/ml; and MAb 110.5 (*24*), 1:10 dilution of ascites fluid. Each set of data is from an experiment on a single virus stock; similar data were obtained with other stocks (*12*). Where shown, SD values are for *n* equals 3 or 4.

Treatment	Virion-bound gp120 (%)	Virion-bound p24 (%)
RF	81 ± 3	86 ± 3
RF + sCD4	17 ± 3	86 ± 3
RF + sCD4 + OKT4A	64	88
RF + sCD4 + Q425	20	85
RF + V1-sCD4	11 ± 4	86 ± 1
RF + 536	77	85
IIIB	68	83
IIIB + sCD4	24	82
IIIB + sCD4 + Q425	27	81
IIIB + sCD4 + 110.5	27	76
IIIB + 110.5	64	77
MN	41	49
MN + sCD4	21	48

that the latter is true. The resistant gp120 moieties may be present on the virion surface as uncleaved gp160 or as disulfide-linked gp120-gp41 complexes; however, we have been unable to detect gp160 in our virion preparations, and others have also described the absence of gp160 from virions (*17, 20*).

The V1 domain of sCD4 (*8*), which contains the gp120 binding site (*21*), was sufficient to dissociate the gp120-gp41 complex (Table 1); regions of sCD4 outside the V1 domain therefore do not participate in this process. No significant shedding of gp120 from HTLV-III_{RF} was induced by binding of the MAb 536 (*22*) to a region of gp120 implicated in CD4 binding (*2*). This antibody competitively inhibits the sCD4-gp120 interaction (*13, 22*) and neutralizes infectivity (*22*); therefore, the binding of a macromolecule close to the "CD4 binding site" is not sufficient to trigger the conformational change necessary for dissociation of gp120 from gp41. Similarly, MAbs Q425 (to the third domain of CD4) (*23*) and 110.5 (to the V3 loop of gp120) (*24*), both of which neutralize HIV-1 at a stage subsequent to CD4 binding, did not inhibit sCD4-induced shedding. Nor did MAb 110.5 binding to HTLV-III_B virions cause gp120 to dissociate (Table 1).

We assessed whether gp120 dissociation from gp41 in response to sCD4 binding could occur on the cell surface as well as on virions. When sCD4 was added to H9 cells chronically infected with HTLV-III_B, to 8E5 cells (*25*) containing a defective HTLV-III_B provirus, or to CHO cells expressing gp120-gp41, gp120 was slowly secreted into the medium (Fig. 3). The shedding of gp120 from the cells was unaffected by 10 mM sodium azide, suggesting that metabolic activity was not required. The rate of sCD4-induced gp120 shedding from the cell surface was slower than from virions, and higher sCD4 concentrations were required (Fig. 3). One possible explanation for this is that sCD4 may efficiently disrupt the gp120-gp41 association only on mature virions. Consistent with this interpretation is the observation that a greater concentration of sCD4 was required to induce shedding of gp120 from transfected CHO cells (which do not contain viral particles) than from H9 and 8E5 cells. Maturation of the HIV virion may, therefore, partially destabilize the gp120-gp41 complex (*18*). The existence of differences between the mechanisms of virus-cell and cell-cell fusion is implied by recent data from Camerini and Seed (*26*). The observation that gp120-gp41 dissociation induced by sCD4 occurs much more slowly and requires higher sCD4 concentrations on the cell surface than on the virion surface may be relevant to these differences.

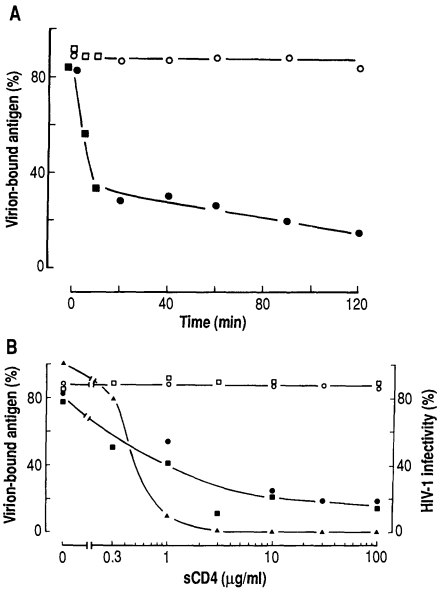

Fig. 2. Shedding of gp120 from virions induced by sCD4. (**A**) HIV-1 (HTLV-III_{RF}) was incubated at 37°C for the times indicated with sCD4 (100 μg/ml) before chromatography on Sephacryl S-1000 (*9*). The peaks corresponding to infectious virus (0.6 to 1.2 ml) and soluble antigen (1.2 to 2.4 ml) were collected and assayed for gp120 and p24 (*9, 10*). The percentages of the total recovered gp120 (●, ■) and p24 (○, □) that were present in the virus peak are shown. The total recoveries of gp120 and p24 from each column varied by ±50%. Data from two experiments on the same virus stock are shown, and were similar to those derived from several experiments carried out with other virus stocks (HTLV-III_B and HTLV-III_{RF}). (**B**) HIV-1 (HTLV-III_{RF}) was incubated for 90 min at 37°C with sCD4 at the concentrations indicated before determination of the percentages of gp120 (●, ■) and p24 (○, □) that were virion bound. Data from two experiments with the same virus stock are shown, in one of which the amount of infectious HIV-1 present at each sCD4 concentration was also determined (*9*) (▲). The $TCID_{50}$ values for 50-μl samples corresponding to the data points were $10^{5.7}$, $10^{5.6}$, $10^{4.7}$, $10^{2.9}$, $10^{2.2}$, $10^{1.2}$, and $10^{1.2}$ and the data are plotted as percentages of the infectivity ($10^{5.7}$ = 100%) without sCD4. Similar data were also obtained with other virus stocks (HTLV-III_B and HTLV-III_{RF}).

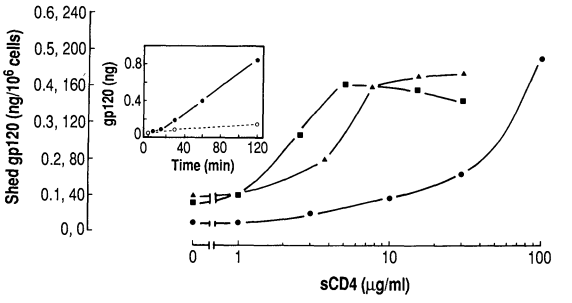

Fig. 3. Shedding of gp120 from cell surfaces induced by sCD4. CHO cells expressing recombinant gp120-gp41 (HTLV-III_B) (●) 8E5 cells containing a *pol*-defective HIV-1 virus (*25*) (■), and H9 cells infected with HIV-1 (HTLV-III_B) (▲) were incubated at a density of 10^7 cells/ml for 2 hours at 37°C with the sCD4 concentrations indicated. The supernatant media were treated with 1% Empigen to disrupt and inactivate virions (*13*) and then assayed for gp120 by ELISA (*9, 10*). The right-hand abscissa (0 to 240) refers to H9 cells, the left-hand (0 to 0.6) to CHO cells and 8E5 cells. (**Inset**) 8E5 cells were incubated at 37°C with (●) or without (○) sCD4 (5 μg/ml) for the times indicated before assay of the supernatant medium for gp120. The data shown are from experiments representative of several of similar design.

We and others (*19*) have shown that sCD4 binding dissociates gp120 from gp41 on virions and HIV-1–infected cells. We suggest that a similar reaction takes place when HIV-1 binds to CD4 on the cell surface and that the dissociation of the gp120-gp41 complex exposes the fusigenic domain on gp41. In the context of the cell surface, however, other processes preceding or subsequent to this reaction may also be necessary: the freedom of gp120 to dissociate from gp41 may be greater when sCD4 binds in solution to the virion than when the ternary complex of CD4-gp120-gp41 forms between the viral and cell membranes. It is at this point in the fusion process that the small population of virion gp120 molecules that do not dissociate from gp41 on CD4 binding may have a role: we speculate that it is through these molecules that the virion remains attached to the cell surface as the fusion reaction takes place.

The sCD4-induced shedding of gp120 is likely to increase the exposure of the gp41 fusigenic domain on the virus surface, although we have no direct evidence for this. A priori, this might be expected to increase the fusion potential of HIV-1, but we suggest that premature exposure of the fusigenic domain inactivates the fusion mechanism. An analogous situation has been demonstrated with many pH-dependent enveloped viruses, whose ability to fuse with host cells is rapidly and irreversibly inactivated by treatment with acid medium before the virus-cell interaction (*27*). Alternatively, virions with an exposed fusigenic domain may indeed have increased infectivity but we are unable to detect this because of the competitive inhibition by sCD4 of HIV-1 binding to the cell surface via its residual gp120 molecules. It may be that increased exposure of the fusigenic domain by sCD4 is responsible for the observation that sCD4 increases the infectivity of simian immunodeficiency virus for some human cell lines

(*28*). Finally, it will be interesting to ascertain whether the mechanism of removal of viral ligands by soluble forms of cell surface receptors is also operative for other viruses, such as for rhinoviruses treated with the soluble intercellular adhesion molecule–1 (ICAM-1) receptor (*29*).

REFERENCES AND NOTES

1. A. G. Dalgleish *et al.*, *Nature* **312**, 763 (1984); D. Klatzmann *et al.*, *ibid.*, p. 767; J. S. McDougal *et al.*, *Science* **231**, 382 (1986).
2. L. A. Lasky *et al.*, *Cell* **50**, 975 (1987).
3. W. G. Robey *et al.*, *Science* **228**, 593 (1985); F. D. Veronese *et al.*, *ibid.* **229**, 1402 (1985); J. M. McCune *et al.*, *Cell* **53**, 55 (1988).
4. B. S. Stein *et al.*, *Cell* **49**, 659 (1987); M. O. McClure, M. Marsh, R. A. Weiss, *EMBO J.* 7, 513 (1988).
5. M. Marsh and R. Helenius, *Adv. Virus Res.* **36**, 107 (1989).
6. W. R. Gallagher, *Cell* **50**, 327 (1987); M. L. Kowalski *et al.*, *Science* **237**, 1351 (1987); F. Gonzalez-Scarano, M. N. Waxham, A. M. Ross, J. A. Hoxie, *AIDS Res. Hum. Retroviruses* **3**, 245 (1987); M. L. Bosch *et al.*, *Science* **244**, 694 (1989).
7. K. C. Deen *et al.*, *Nature* **331**, 82 (1988).
8. J. Arthos *et al.*, *Cell* **57**, 469 (1989).
9. Chronically infected H9 cells were cultivated with uninfected H9 cells (1:4), and the medium was harvested 48 hours after infection. We had previously determined that this regimen was optimal for the production of virus with high infectious titers in which a large percentage of the gp120 and p24 in the cultures was virion bound (*12*). Samples of supernatant virus (100 μl) were fractionated by gel-exclusion chromatography under containment conditions on 2-ml columns of Sephacryl S-1000 (Pharmacia). Protein was eluted from the column with tris-buffered saline (TBS) (*10*), and fractions (two drops; average volume, 85 μl) were collected. The infectious HIV-1 content was assessed as described [J. A. McKeating *et al.*, *J. Gen. Virol.* **70**, 3327 (1989)]. We detected gp120 by twin-site ELISA (*10*). The capture antibody was D7324 (Aalto BioReagents, Dublin, Eire) to the conserved COOH-terminus of gp120, and bound gp120 was detected with a pool of HIV-1–positive human serum, an alkaline-phosphatase conjugated antibody to human immunoglobulin G (IgG) (SeraLab, Crawley, U.K.), and AMPAK (Novo Nordisk, Cambridge, U.K.) (*10*). The assays were calibrated with recombinant (CHO cell) gp120 (Celltech) [J. P. Moore *et al.*, *AIDS* **4**, 307 (1990)]. In some assays R1/87 rabbit antiserum (*10*) to recombinant gp120 ($HTLV\text{-}III_B$) was used instead of human HIV-1–positive serum, and similar results were obtained. Shed gp120 was shown to be present in a complex with sCD4 by detection of sCD4 bound onto antibody D7324–captured gp120 (*12, 13*); sCD4 does not interfere with the gp120 assays used. We measured p24 by twin-site ELISA with D7320 (Aalto BioReagents) as the capture antibody, alkaline-phosphatase conjugated MAb EH12E1 [R. B. Ferns *et al.*, *ibid.* **3**, 829 (1989)] as detection antibody, and AMPAK. The *gag* epitopes recognized by antibodies D7320 and EH12E1 are conserved between HIV-1 strains. The assay was calibrated with recombinant p24 (J. E. M. Gilmour *et al.*, *ibid.*, p. 717). The gp120 and p24 assays can detect approximately 3 pg of recombinant protein in 100 μl, and both are specific; recombinant gp120 is not detected in the p24 assay and vice versa. Furthermore, there was no ELISA signal when an irrelevant antibody [D7335 to the COOH-terminus of HIV type–2 (HIV-2) gp120] was substituted for the capture antibody in either assay.
10. J. P. Moore and R. F. Jarrett, *AIDS Res. Hum. Retroviruses* **4**, 369 (1988); J. P. Moore, L. A. Wallace, E. A. C. Follett, J. A. McKeating, *AIDS* **3**, 155 (1989).
11. H. R. Gelderblom *et al.*, *Virology* **156**, 171 (1987).
12. J. P. Moore, J. A. McKeating, R. A. Weiss, Q. J. Sattentau, unpublished data; J. P. Moore, J. A. McKeating, W. A. Norton, Q. J. Sattentau, *J. Virol.*, in press.
13. J. P. Moore, *AIDS* **4**, 297 (1990).
14. R. A. Byrn *et al.*, *J. Virol.* **63**, 4370 (1989).
15. S. P. Layne *et al.*, *Nature* **346**, 277 (1990).
16. M. Schawaller, G. E. Smith, J. J. Skehel, D. C. Wiley, *Virology* **172**, 367 (1989).
17. A. Pinter *et al.*, *J. Virol.* **63**, 2674 (1989).
18. H. R. Gelderblom, H. Reupke, G. Pauli, *Lancet* **ii**, 1016 (1985); J. Schneider, O. Kaaden, T. D. Copeland, S. Oroszlan, G. Hunsmann, *J. Gen. Virol.* **67**, 2533 (1986).
19. R. Kirsh *et al.*, *AIDS Res. Hum. Retroviruses* **6**, 1215 (1990).
20. R. L. Willey, J. S. Bonafacino, B. J. Potts, M. A. Martin, R. D. Klausner, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 9580 (1988).
21. Q. J. Sattentau and R. A. Weiss, *Cell* **52**, 631 (1988).
22. N.-C. Sun *et al.*, *J. Virol.* **63**, 3579 (1989).
23. D. Healey *et al.*, *J. Exp. Med.* **172**, 1233 (1990).
24. P. S. Linsley, J. A. Ledbetter, E. Kinney-Thomas, S.-L. Hu, *J. Virol.* **62**, 3695 (1988).
25. T. M. Folks *et al.*, *J. Exp. Med.* **164**, 280 (1986).
26. D. Camerini and B. Seed, *Cell* **60**, 747 (1990).
27. J. Skehel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 968 (1982); R. W. Doms, A. Helenius, J. White, *J. Biol. Chem.* **260**, 2973 (1985); M. C. Kielian and A. Helenius, *J. Cell Biol.* **107**, 2284 (1985).
28. J. S. Allan, J. Strauss, D. W. Buck, *Science* **247**, 1084 (1990).
29. S. D. Marlin *et al.*, *Nature* **344**, 70 (1990).

30. We thank R. W. Sweet for sCD4 and the purified V1 domain of sCD4; P. E. Stephens for CHO cells expressing gp120-gp41; D. D. Ho for MAb 536; R. B. Ferns and R. S. Tedder for MAb EH12E1; E. Kinney-Thomas for MAb 110.5; R. S. Daniels and J. J. Skehel for R1/87 serum; S. E. Adams for recombinant HIV-1 p24; H. Holmes for AIDS Directed Programme reagents; R. C. Gallo for virus isolates; R. Axel, P. R. Clapham, and T. F. Schulz for suggestions; and A. McKnight for assistance. Supported by the U.K. Medical Research Council AIDS Directed Programme and by the Cancer Research Campaign.

24 May 1990; accepted 24 August 1990

Contributions of Two Types of Calcium Channels to Synaptic Transmission and Plasticity

BRIAN EDMONDS,* MARC KLEIN,† NICHOLAS DALE,‡ ERIC R. KANDEL

In *Aplysia* sensory and motor neurons in culture, the contributions of the major classes of calcium current can be selectively examined while transmitter release and its modulation are examined. A slowly inactivating, dihydropyridine-sensitive calcium current does not contribute either to normal synaptic transmission or to any of three different forms of plasticity: presynaptic inhibition, homosynaptic depression, and presynaptic facilitation. This current does contribute, however, to a fourth form of plasticity—modulation of transmitter release by tonic depolarization of the sensory neuron. By contrast, a second calcium current, which is rapidly inactivating and dihydropyridine-insensitive, contributes to release elicited by the transient depolarization of an action potential and to the other three forms of plasticity.

CALCIUM HAS AN UNUSUAL ROLE IN nerve and muscle cells in that it is both a carrier of positive charge that contributes to excitability and an intracellular second messenger for a variety of cellular responses, including contraction and secretion (*1*). Calcium influx into most neurons occurs through at least two of three classes of voltage-dependent channels (*2–6*): an L-type channel that inactivates slowly (*7*) and is modulated by dihydropyridine compounds; and at least one of two dihydropyridine-insensitive channels, an N type that is activated by strong depolarization, or a T type activated by weak depolarization. What role does each of these channels have in the release of chemical transmitter and in synaptic plasticity?

In rat sympathetic neurons, the release of norepinephrine is primarily dependent on the N-type Ca^{2+} channel and does not appear to require the L-type channel (*8, 9*). By contrast, in dorsal root ganglion neurons from chicks, L-type channels contribute to the release of substance P (*8, 10*). However, in studies of vertebrate cells, transmitter release could not be initiated physiologically by action potentials, because dihydropyridine antagonists of L-type channels are only effective in tonically depolarized cells (*3, 10, 11*). As a result, release was elicited by prolonged depolarization of cells with a solution containing elevated concentrations of K^+. Moreover, release was monitored not by recording discrete postsynaptic potentials (PSPs) in a follower neuron but by determining the amount of labeled transmitter that accumulated in the bathing solution. As a result, the normal (fast) kinetics of release

Howard Hughes Medical Institute, Center for Neurobiology and Behavior, Columbia University, College of Physicians and Surgeons, New York, NY 10032.

*Present address: Department of Pharmacology, University College of London, London LOCIE 6BT, United Kingdom.
†Present address: Clinical Research Institute of Montreal, Montreal, Quebec, Canada, H2W 1R7.
‡Present address: Department of Zoology, University of Bristol, Bristol BS8 1UG, United Kingdom.