Immunotherapeutic strategies for EAE have included infusion of monoclonal antibodies (MAbs) to Vβ8, which prevents disease in mice (*5, 6*) and reverses it if administered after onset (*5*). Similarly, infusion of a MAb to a clonotypic determinant on the surface of encephalitogenic T cells protects Lewis rats from EAE (*22*). Resistance to EAE induction has also been conferred by vaccination with attenuated, encephalitogenic T cell clones (*23, 24*) and by transfer of ex vivo–derived immunoregulatory T cells (*18, 19, 25*).

Vaccination with TCR peptides is preferable to MAb infusion because persistent, active immunity is induced. Whereas active immunity is also induced by vaccinating with attenuated encephalitogenic T cells, the efficacy of this procedure is reported to be variable (*26*). Further, whole T cell vaccination induces immunity to T cell determinants other than those that confer protection (*26*) and, in an outbred population, must be administered autologously to avoid alloreactive complications. Administration of ex vivo–derived regulatory T cells must be similarly individualized. Such labor-intensive strategies are unlikely to be employed on a significant scale. TCR peptide vaccination is a selective approach to the modulation of T cell–mediated autoimmunity. It also may be applicable to the helper T cells in antibody-mediated autoimmunity, T cell lymphomas, and other pathogenic conditions mediated by specific, oligoclonal T cell populations.

REFERENCES AND NOTES

1. A. Ben-Nun, H. Wekerle, I. R. Cohen, *Eur. J. Immunol.* **11**, 195 (1981).
2. A. Ben-Nun and I. R. Cohen, *J. Immunol.* **129**, 303 (1982).
3. S. Zamvil *et al.*, *Nature* **317**, 355 (1985).
4. S. S. Zamvil *et al.*, *J. Exp. Med.* **167**, 1586 (1988).
5. H. Acha-Orbea *et al.*, *Cell* **54**, 163 (1988).
6. J. L. Urban *et al.*, *ibid.*, p. 577.
7. R. R. Burns *et al.*, *J. Exp. Med.* **169**, 27 (1989).
8. J. Chluba, C. Steeg, A. Becker, H. Wekerle, J. Epplen, *Eur. J. Immunol.* **19**, 279 (1989).
9. E. Heber-Katz and H. Acha-Orbea, *Immunol. Today* **10**, 164 (1989).
10. S. S. Zamvil *et al.*, *Nature* **324**, 258 (1986).
11. C.-H. J. Chou, R. B. Fritz, F. C.-H. Chou, R. F. Kibler, *J. Immunol.* **123**, 1540 (1979).
12. R. K. Wilson, E. Lai, P. Concannon, R. K. Barth, L. E. Hood, *Immunol. Rev.* **101**, 149 (1988).
13. R. A. C. Hughes and H. C. Powell, *J. Neuropath. Exp. Neurol.* **43**, 154 (1984).
14. J. A. Berzofsky *et al.*, *Immunol. Rev.* **98**, 9 (1987).
15. J. B. Rothbard and W. R. Taylor, *EMBO J.* **7**, 93 (1988).
16. A. R. M. Townsend *et al.*, *Cell* **44**, 959 (1986).
17. M. D. Howell, unpublished data.
18. D. Sun, Y. Qin, J. Chluba, J. T. Epplen, H. Wekerle, *Nature* **332**, 843 (1988).
19. D. Sun, A. Ben-Nun, H. Wekerle, *Eur. J. Immunol.* **18**, 1993 (1988).
20. D. Sun, M. D. Howell, S. W. Brostoff, unpublished data.
21. S. Buus, A. Sette, S. M. Colon, H. M. Grey, *Science* **242**, 1045 (1988).
22. M. Owhashi and E. Heber-Katz, *J. Exp. Med.* **168**, 2153 (1988).
23. A. Ben-Nun, H. Wekerle, I. R. Cohen, *Nature* **292**, 60 (1981).
24. O. Lider, T. Reshef, E. Beraud, A. Ben-Nun, I. R. Cohen, *Science* **239**, 181 (1988).
25. K. E. Ellerman, J. M. Powers, S. W. Brostoff, *Nature* **331**, 265 (1988).
26. H. Offner, R. Jones, B. Celnik, A. A. Vandenbark, *J. Neuroimmunol.* **21**, 13 (1989).
27. W. L. Hays, *Statistics* (Holt, Rinehart & Winston, New York, 1981), pp. 552–555.

28. We thank H. Wekerle for communication of data prior to publication, K. Ellerman for the MBP-reactive Lewis rat T cell line, D. Sun for determining the proliferative response of anti-S1 to TCR β chain peptides, and A. Esty for reviewing the manuscript.

15 August 1989; accepted 15 September 1989

The Neuron-Specific Protein PGP 9.5 Is a Ubiquitin Carboxyl-Terminal Hydrolase

KEITH D. WILKINSON,* KEUNMYOUNG LEE, SEEMA DESHPANDE, PENELOPE DUERKSEN-HUGHES, JEREMY M. BOSS, JAN POHL

A complementary DNA (cDNA) for ubiquitin carboxyl-terminal hydrolase isozyme L3 was cloned from human B cells. The cDNA encodes a protein of 230 amino acids with a molecular mass of 26,182 daltons. The human protein is very similar to the bovine homolog, with only three amino acids differing in over 100 residues compared. The amino acid sequence deduced from the cDNA was 54% identical to that of the neuron-specific protein PGP 9.5. Purification of bovine PGP 9.5 confirmed that it is also a ubiquitin carboxyl-terminal hydrolase. These results suggest that a family of such related proteins exists and that their expression is tissue-specific.

COVALENT ATTACHMENT OF UBIQUITIN to a number of cellular proteins has been postulated to play a role in a variety of cellular processes (*1–3*). This widespread posttranslational modification of proteins is thought to target the attached protein for various metabolic fates. In the case of proteolysis, polyubiquitination of substrate proteins is observed, and these conjugates are recognized and degraded by specific proteases in the cell (*2*). In contrast, the reversible ubiquitination of histones is limited to one or two ubiquitin molecules per histone molecule, does not lead to degradation, and may be important for chromatin condensation or cell cycle progression (*4–6*). Covalent attachment of ubiquitin to the T cell homing receptor (*7*) and the platelet-derived growth factor receptor (*8*) has been demonstrated, but the consequences of this modification are unknown. Three eukaryotic genes code for proproteins that consist of an NH_2-terminal ubiquitin and a COOH-terminal ribosomal protein (*9, 10*); the ubiquitin is proteolytically processed from these proteins at a poorly defined point in the maturation of the ribosome. Finally, whereas a fourth ubiquitin gene codes for a polyubiquitin precursor that is a heat shock protein (*11, 12*), its role in the stress response is undefined. These diverse functions have one common feature; that is, they all require that ubiquitin derivatives be recognized by specific proteases that are able to hydrolyze a peptide bond at the COOH-terminal glycine of ubiquitin. Thus, the ubiquitinyl-*N*ε amide bond is enzymatically hydrolyzed during the removal of ubiquitin from histones that occurs before mitosis (*13, 14*), cytoplasmic conjugates are disassembled by activities present in most tissues (*15*), and proteolytic intermediates must be released from the COOH-terminus

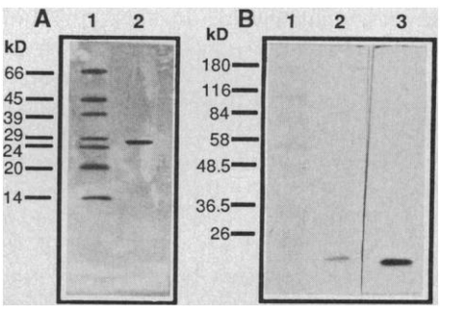

Fig. 1. Specificity of affinity-purified antibodies to homogeneous UCH-L3. Antibodies were affinity-purified with antigen immobilized on nitrocellulose paper. (**A**) Silver-stained SDS-polyacrylamide gel showing the purified UCH-L3 used to elicit antibodies in rabbits. Lane 1, molecular mass standards; lane 2, purified UCH-L3. (**B**) Protein immunoblots of an SDS-polyacrylamide gel obtained using the affinity-purified antibody. Lane 1, molecular mass standards; lane 2, bovine thymus homogenate; lane 3, purified bovine UCH-L3.

K. D. Wilkinson, K. Lee, S. Deshpande, P. Duerksen-Hughes, Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322.
J. M. Boss, Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322.
J. Pohl, Emory University Microchemical Facility, Emory University School of Medicine, Atlanta, GA 30322.

*To whom correspondence should be addressed.

of ubiquitin to allow its regeneration for another catalytic cycle (*15*). Similarly, enzymes must be present that hydrolyze the ubiquitinyl-*N*α amide bond during processing of the polyubiquitin and the ubiquitin COOH-terminal extension proteins coded by the known ubiquitin genes.

With the use of a generic substrate, the COOH-terminal ethyl ester of ubiquitin (*16*), four such enzymatic activities have been separated and identified from bovine thymus (*17*). All are thiol proteases and have high-affinity binding sites for ubiquitin, and three of the four have apparent molecular masses of ~30 kD. We have isolated the predominant bovine thymus isozyme (UCH-L3) (*18*), determined the amino acid sequence of over 40% of it, and raised antibodies to it. Analysis of the purified bovine protein by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining revealed a single band with an apparent molecular mass of 26,000 ± 500 daltons (Fig. 1A). Polyclonal antibodies to this protein were raised in rabbit and then affinity-purified. A major immunoreactive band of the appropriate molecular mass was observed (Fig. 1B) when this antibody was used in immunoblots of either the purified protein or thymus homogenates.

With the use of the specific antibodies to the bovine thymus isozyme, we have cloned the corresponding cDNA sequence from a human B cell cDNA library (*19*) (Fig. 2). This sequence appears to be full length (*20*) and to code for UCH-L3 on the basis of several lines of evidence. First, an open reading frame of 690 bp is present in the middle of this sequence and could code for a protein of the appropriate size and isoelectric point, as determined for bovine UCH-L3. Second, the fusion protein coded by this cDNA sequence cross-reacts with the antibodies to purified bovine UCH-L3. Finally, the fusion protein encoded by this insert is enzymatically active and able to hydrolyze ubiquitin ethyl ester (*21*). To confirm this assignment, we have obtained the amino acid sequences of ten tryptic peptides from the bovine protein (Fig. 2). A comparison of the deduced human protein sequence with the bovine peptide sequences demonstrates that there is a high degree of similarity. Of the 100 residues determined by amino acid sequencing, only two differ from the corresponding human sequence; Val^{127} of the human protein was determined to be an Ala in the bovine protein (a C to T change at the nucleotide level) and Ser^{161} was determined to be an Asn (a G to A change). In addition, we obtained a bovine peptide corresponding to residues 103 to 108 of the human protein by tryptic digestion. Residue 102 is a His in the human protein, but can be inferred to be a Lys or, more likely, an Arg (*22*) in the bovine protein because tryptic cleavage occurred at this position.

A search of the Protein Identification Resource Data Bank using the deduced human UCH-L3 amino acid sequence showed similarity with only one protein, the human neuron-specific cytoplasmic protein PGP 9.5 (*23*). In addition, the sequence of a yeast ubiquitin hydrolase has been reported (*24*). The two human proteins have 126 identical residues and 35 conserved changes. The yeast sequence is most similar to UCH-L3 (73 identities), and 45 residues are conserved in all three sequences (Fig. 3). Clearly, these are related proteins and it was of interest to determine if the neuronal protein exhibits ubiquitin COOH-terminal hydrolytic activity.

PGP 9.5 is among the most abundant proteins of brain, constituting from 1 to 5% of the soluble protein. It is a cytoplasmic protein of 212 amino acids (*23*) that has been localized to neuronal and diffuse neuroendocrine tissues in a variety of vertebrate organisms (*25*). Immunohistological studies demonstrate that it is localized in the neurons and ganglia, but not other cell types in the brain, and must therefore be even

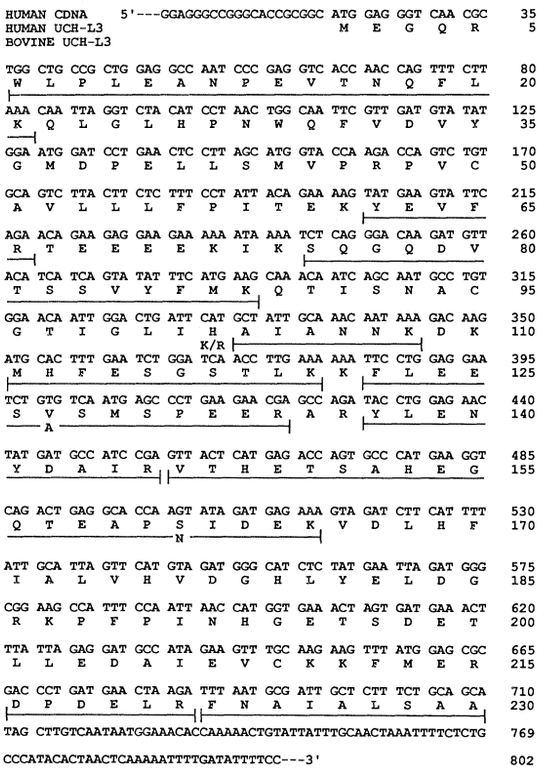

Fig. 2. The covalent structure of UCH-L3. The top line shows the human UCH-L3 cDNA sequence and the second line gives the deduced amino acid (*35*) sequence coded by this cDNA. The sequences of ten bovine peptides are delineated (|–|) on the bottom line. Differences in the amino acid sequence between the human and bovine sequences occur at positions 101, 127, and 161. The cDNA insert was excised from the λgt11 clone by cleavage with Eco RI and subcloned into the Eco RI restriction site of pSP72. The plasmid insert was digested with Eco RI, Kpn I, and Bgl II, and the resulting fragments were subcloned into pSP72 that had been treated with Eco RI and Bgl II (284-bp insert), Eco RI and Kpn I (163-bp insert), or Kpn I and Bgl II (362-bp insert). The double-stranded DNA inserts were sequenced using DNA Sequenase Kit Version 2 (United States Biochemical Corp.). Tryptic digestion of bovine UCH-L3 and purification of the peptides was performed as described (*36*) except that urea was not present during the digestion. The peptides were sequenced using an Applied Biosystems 477A-120A sequencing system.

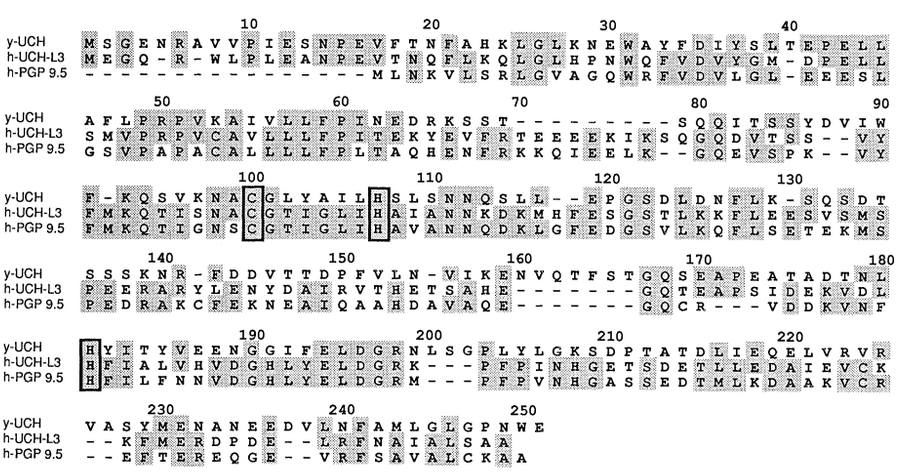

Fig. 3. Similarity of the deduced amino acid sequences of yeast UCH, human UCH-L3, and human PGP 9.5. Identical residues are shaded and the conserved Cys and His residues are outlined.

more abundant in these cell types. The function of PGP 9.5 is unknown, but its tissue distribution parallels that of another neuronal marker, neuron-specific enolase. Fractions obtained in the last two steps of the purification of bovine PGP 9.5 (*26*) were assayed for ubiquitin COOH-terminal hydrolase activity (*16*). The enzymatic activity detected in these preparations copurified with PGP 9.5 in both the gel filtration (Fig. 4A) and ion exchange (Fig. 4B) steps of the preparation. The purified preparation exhibited a ubiquitin COOH-terminal hydrolase specific activity of 4 units per milligram.

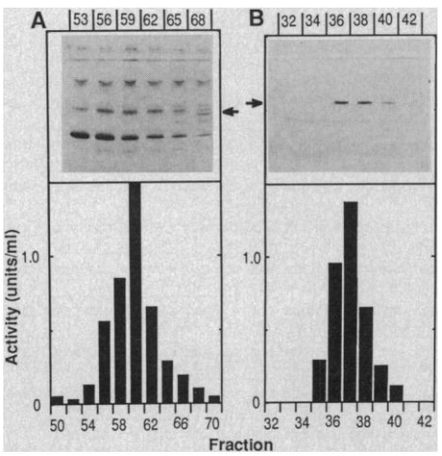

Fig. 4. Copurification of bovine PGP 9.5 and ubiquitin COOH-terminal hydrolase activity. (**A**) UCH activity (lower) and SDS-PAGE (upper) analysis of fractions obtained after the gel filtration step of purification. (**B**) UCH activity (lower) and SDS-PAGE (upper) analysis of fractions obtained after the DEAE–ion exchange step of purification. The arrows indicate the migration position of PGP 9.5.

Table 1. Alignment of tryptic peptide sequences from bovine UCH-L1 and bovine PGP 9.5 with the deduced amino acid sequence of human PGP 9.5. The one-letter code is used to specify the amino acids (*35*), where X indicates an unidentified residue. Purified bovine PGP 9.5 and UCH-L1 (*27*) were digested and purified by the procedures described previously (*36*). Selected peptides were sequenced by the Emory University Microchemical Facility. The peptides sequences obtained were aligned with the deduced amino acid sequence of human PGP 9.5 and the amino acid residue number based on that sequence is shown.

Peptide sequences		Human PGP 9.5 deduced residue numbers
PGP 9.5	UCH-L1	
LGVAGQXR	LGVAGQWR	9–16
GQEVSPK		61–67
VYFMK		68–72
	MPFPVN	168–173
VCR		189–191
EFTER		192–196
FSAVALCX		203–210

This is to be compared to the specific activity of 10 units per milligram for UCH-L3 catalyzing the same reaction. Thus, it is unlikely that the enzymatic activity in the purified PGP 9.5 preparation was due to a small amount of contaminating protein.

Several lines of evidence suggest that PGP 9.5 corresponds to ubiquitin COOH-terminal hydrolase isozyme L1, which has recently been affinity-purified from bovine thymus (*27*). First, the physical properties of these two proteins (PGP 9.5 and isozyme L1) are very similar; both elute from DEAE-cellulose at 0.1*M* NaCl and exhibit apparent molecular masses of ~24,500 daltons on SDS-PAGE. Second, the kinetic properties of PGP 9.5, including a low Michaelis constant (K_m) for ubiquitin and a sensitivity to thiol reagents and metal ions (*28*), correspond most closely to isozyme L1. Finally, we have obtained the amino acid sequence of six tryptic peptides from bovine brain PGP 9.5 and two from affinity-purified bovine thymus isozyme L1 (Table 1). All of these peptides are consistent with those predicted by the cDNA sequence of human PGP 9.5. In addition, one peptide, corresponding to residues 9 to 16 of human PGP 9.5, was found in both bovine PGP 9.5 and bovine isozyme L1.

The covalent structures of the two human enzymes UCH-L3 and PGP 9.5 and the yeast enzyme (*24*) define important regions in this class of enzymes. The active site Cys must correspond to the only conserved Cys, residue 100 using the numbering system of the alignments shown in Fig. 3. Catalytically essential His residues must be either of two conserved His residues at positions 107 and 181 of the aligned sequences. Elements forming the ubiquitin binding site might also be highly conserved and candidate regions that might participate in this interaction include residues 43 to 60 and 190 to 198.

Thus, the expression of ubiquitin COOH-terminal hydrolase isozymes appears to be tissue-specific, suggesting that the role of ubiquitin may vary with tissue type or differentiation. These isozymes may participate in disassembling ubiquitin-protein conjugates formed during normal protein metabolism. If so, the expression of isozyme L1 may be altered in various neurological disease states. Several such neurodegenerative diseases, such as Alzheimer's disease (*29, 30*), Parkinson's disease (*31, 32*), progressive supranuclear palsy (*33*), and Pick's and Kufs' disease (*34*), are known to result in neurofibrillary tangles, which are rich in ubiquitinated proteins. A defect or alteration in the expression of enzymes catalyzing the removal of ubiquitin from such conjugates may be a characteristic of these disease states.

REFERENCES AND NOTES

1. K. D. Wilkinson, *Anticancer Drug Design* **2**, 211 (1987).
2. M. Rechsteiner, *Annu. Rev. Cell Biol.* **3**, 1 (1987).
3. A. Hershko and A. Ciechanover, *Prog. Nucleic Acid Res. Mol. Biol.* **33**, 19 (1986).
4. D. Finley, A. Ciechanover, A. Varshavsky, *Cell* **37**, 43 (1984).
5. H. Busch and I. L. Goldknopf, *Mol. Cell. Biochem.* **40**, 173 (1981).
6. R. S. Wu, K. W. Kohn, W. M. Bonner, *J. Biol. Chem.* **256**, 5916 (1981).
7. T. St. John *et al.*, *Science* **231**, 845 (1986).
8. Y. Yarden *et al.*, *Nature* **323**, 226 (1986).
9. D. Finley, B. Bartel, A. Varshavsky, *ibid.* **338**, 394 (1989).
10. K. L. Redman and M. Rechsteiner, *ibid.* p. 438.
11. K. Tanaka, K. Matsumoto, A. Toh-e, *EMBO J.* **7**, 495 (1988).
12. U. Bond and M. J. Schlesinger, *Mol. Cell Biol.* **6**, 4602 (1986).
13. S. Matsui, A. A. Sandberg, S. Negoro, B. K. Seon, G. Goldstein, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1535 (1982).
14. I. L. Goldknopf, S. Sudhakar, F. Rosenbaum, H. Busch, *Biochem. Biophys. Res. Commun.* **95**, 1253 (1980).
15. A. Hershko, A. Ciechanover, H. Heller, A. L. Haas, I. A. Rose, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1783 (1980).
16. K. D. Wilkinson, M. J. Cox, A. N. Mayer, T. Frey, *Biochemistry* **25**, 6644 (1986).
17. A. N. Mayer and K. D. Wilkinson, *ibid.* **28**, 166 (1989).
18. Bovine UCH-L3 was isolated as described (*17*), followed by affinity chromatography on ubiquitin-Sepharose and gel filtration [I. A. Rose and J. V. Warms, *Biochemistry* **22**, 4234 (1983)].
19. A series of human cell lines were screened to determine an appropriate source for a cDNA library. Protein immunoblots demonstrated that the antibodies cross-react with a human protein of the correct molecular mass and which is present in significant amounts in most human T and B cell lines tested, as well as in HL60 cells and erythroblasts. On the basis of this result, it appears that the antigenic epitopes are conserved from bovine to humans. Accordingly, a human B cell cDNA library in λgt11 was screened using immunological detection of the fusion protein products to identify the appropriate clones.
20. The cDNA sequence obtained is most likely a full-length sequence. All bovine peptides sequenced corresponded to homologous peptides in the deduced human protein sequence. We have determined the amino acid sequence of over 40% of the bovine protein and might have expected to observe other sequences if there was a significantly larger preprotein or additional residues at the NH_2-terminus; as the NH_2-terminus is blocked however, we could not directly demonstrate this. The apparent molecular mass of the mature bovine protein determined by SDS-PAGE is 26,000 ± 500 daltons, in good agreement with the predicted molecular mass of 26,182 daltons. Finally, the COOH-terminus is confirmed by the finding of a bovine tryptic peptide corresponding to residues 222 to 230 of the human sequence that terminated in Ala.
21. Four antigen-positive clones were plaque-purified and the β-galactosidase fusion protein was prepared from each [T. Maniatis, E. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982)]. These fusion protein preparations were tested for ubiquitin COOH-terminal hydrolase activity. One clone expressed a fusion protein with significant enzymatic activity (7.5×10^{-4} units per milligram of protein) whereas the three other preparations exhibited specific activities of less than 1×10^{-6} units per milligram of protein.
22. The latter change would appear more likely because it requires only an A to G change at the DNA level.
23. I. N. M. Day and R. J. Thompson, *FEBS Lett.* **210**, 157 (1989).
24. H. I. Miller *et al.*, *Biotechnology* **7**, 698 (1989).
25. P. O. G. Wilson *et al.*, *Br. J. Exp. Pathol.* **69**, 91

(1988).
26. PGP 9.5 was isolated from frozen calf brain exactly as described for the human protein [J. F. Doran, P. Jackson, P. A. M. Kynoch, R. J. Thompson, *J. Neurochem.* **40**, 1542 (1983)] except that all buffers contained 10 m*M* β-mercaptoethanol. The yield of protein from this preparation was 18 mg per kilogram of brain and was identical to that of the published preparations of the human protein. The protein preparation was apparently homogeneous as judged by isoelectric focusing, SDS-PAGE, and PAGE under nondenaturing conditions.
27. P. J. Duerksen-Hughes, M. M. Williamson, K. D. Wilkinson, *Biochemistry*, in press.
28. K. D. Wilkinson *et al.*, unpublished data.
29. G. M. Cole and P. S. Timiras, *Neurosci. Lett.* **79**, 207 (1987).
30. G. Perry, R. Friedman, G. Shaw, V. Chau, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3033 (1987).
31. P. G. Galloway, I. Grundke-Iqbal, K. Iqbal, G. Perry, *J. Neuropathol. Exp. Neurol.* **47**, 654 (1988).
32. C. Bancher *et al.*, *ibid.* **48**, 81 (1989).
33. P. G. Galloway, *Neurosci. Lett.* **91**, 148 (1988).
34. S. Love, T. Saitoh, S. Quijada, G. M. Cole, R. D. Terry, *J. Neuropathol. Exp. Neurol.* **47**, 393 (1988).
35. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
36. M. J. Cox, R. Shapira, K. D. Wilkinson, *Anal. Biochem.* **154**, 345 (1986).

3 July 1989; accepted 18 September 1989

Table 1. Accumulation of [^{3}H]IP$_1$ in striate cortical synaptoneurosomes prepared from 5-week-old kittens (*11*). Results are means ± SEM expressed as percentage of basal phosphoinositide turnover.

Agonist	Concentration (μ*M*)	Percentage of basal turnover	*n*
Glutamate	10	108 ± 5	5
	100	168 ± 4	5
Ibotenate	10	372 ± 14	5
	100	435 ± 37	5
NMDA	100	110 ± 7	3
	300	110 ± 4	3
AMPA	300	102 ± 6	3
Carbachol	100	300 ± 34	5

A Biochemical Correlate of the Critical Period for Synaptic Modification in the Visual Cortex

SERENA M. DUDEK AND MARK F. BEAR

Stimulation of phosphoinositide hydrolysis by excitatory amino acids was studied in synaptoneurosomes of kitten striate cortex at several postnatal ages. Ibotenate and glutamate stimulated phosphoinositide turnover during the second and third postnatal months; *N*-methyl-D-aspartate and DL-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) were without effect. The developmental profile of ibotenate-stimulated phosphoinositide turnover parallels the postnatal changes in cortical susceptibility to visual deprivation. The transient increase in ibotenate-stimulated phosphoinositide turnover does not occur in visual cortex of kittens reared in complete darkness.

MANIPULATIONS OF THE VISUAL environment during early postnatal life can lead to profound and long-lasting changes in the functional organization of the visual cortex. For example, temporary closure of one eyelid in kittens renders striate cortex unresponsive to stimulation of the deprived eye (*1, 2*). Responsiveness to the deprived eye can be restored if it is again allowed to view a normal visual environment while the other eyelid is closed (*3*). This form of synaptic plasticity is limited to a finite period of postnatal development referred to as the "critical" or "sensitive" period (*2–5*). Estimates of the length of the critical period have varied, but there is general agreement that sensitivity to brief lid closure begins in kittens at about 3 weeks of age, peaks during the 5th week, and then gradually disappears between 12 and 16 weeks (*2–5*). We report now that this is precisely the period of postnatal development when the excitatory amino acid ibotenate stimulates phosphoinositide hydrolysis in striate cortex. Phosphoinositide turnover leads to the formation of two intracellular second messengers, inositol triphosphate (IP_3) and diacylglycerol (DAG) (*6*). Thus, these data suggest that excitatory synaptic transmission during the critical period is characterized by unique patterns of second messenger activity and that phosphoinositide hydrolysis may play a central role in the experience-dependent modification of visual cortex.

There is evidence that synaptic excitation in the visual cortex depends on activation of excitatory amino acid (EAA) receptors (*7*). One class of EAA receptor is linked to a phosphoinosidase that catalyzes the hydrolysis of phosphatidylinositol-4,5 bisphosphate to form DAG and IP_3 (*8*). Nicoletti *et al.* (*9*) have showed in rat hippocampus that this metabotropic EAA receptor is activated by glutamate, prefers the agonists ibotenate and quisqualate, and is unresponsive to *N*-methyl-D-aspartate (NMDA) and kainate. This site is distinct from the traditional quisqualate receptor; for example, it is not stimulated by DL-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and is not blocked by kynurenic acid. A similar site has been characterized in rat neocortex (*10*). In both rat hippocampus and neocortex the EAA-stimulated phosphoinositide hydrolysis is low at birth, peaks during early postnatal life, and then declines with increasing age. These observations have prompted speculation that this mechanism is involved in developmental plasticity (*9, 10*). This hypothesis would be strengthened considerably if it could be shown that

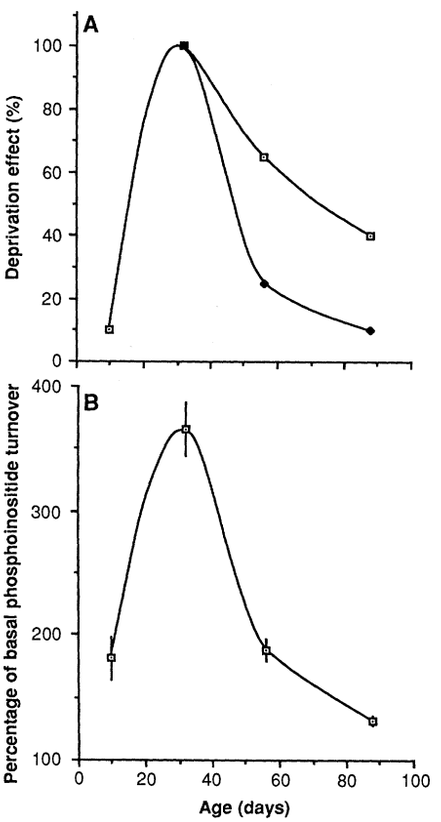

Fig. 1. (**A**) Sensitivity of binocular connections in striate cortex to eyelid suture at different postnatal ages, as estimated by Olson and Freeman by monocular deprivation (⊡) (*2*) and by Blakemore and van Sluyters with the "reverse suture" paradigm (◆) (*3*). The deprivation effect is the percentage of neurons in area 17 with responses dominated by stimulation of the nondeprived eye [data from figure 1 of (*5*)]. (**B**) Phosphoinositide turnover stimulated by 10 μ*M* ibotenate in synaptoneurosomes prepared from kitten striate cortex at different postnatal ages. Data points represent the means ± SEM of at least three experiments, expressed as percentage of basal phosphoinositide turnover.

Center for Neural Science, Brown University, Providence, RI 02912.