Acta Neuropathol (1996) 92 : 49–55

REGULAR PAPER

S. Ahlgren · G. L. Li · Y. Olsson

Accumulation of β-amyloid precursor protein and ubiquitin in axons after spinal cord trauma in humans: immunohistochemical observations on autopsy material

Received: 6 November 1995 / Revised, accepted: 29 December 1995

Abstract We evaluated by immunohistochemistry the presence of β-amyloid precursor protein (ßAPP) and ubiquitin-like material which may accumulate in axons of the human spinal cord subjected to injury. Autopsy material was obtained from nine cases with different types of trauma: breech delivery with neonatal spinal injury, compression of the cord induced by fractures of the vertebral column, haematomas or intradural meningioma. The post-trauma period ranged from 10 days to several years. The spinal cord of six control cases without evidence of injury presented βAPP immunoreactivity in nerve cell bodies and in a few axonal profiles but not in dendrites. Seven of the nine cases with spinal cord trauma showed an accumulation of βAPP-immunoreactive material in axons of the longitudinal tracts at the site of the injury. Five cases presented similar axonal immunoreactivity in the grey matter of the cord. Ubiquitin-like immunoreactivity was present in expanded axons in cases with spinal cord injury. Cases with spinal cord trauma thus present βAPP-immunoreactive axons particularly of the longitudinal tracts in the same way as in trauma to rat spinal cord and in various brain injuries. The aggregation of βAPP-immunoreactive material indicates disturbed axonal transport of βAPP. Accumulation of ubiquitin-like immunoreactive material in expanded axons at the site of trauma may be one prerequisite for degradation of abnormal proteins by the ubiquitin-mediated proteolytic pathway.

Key words β-Amyloid precursor protein · Ubiquitin · Human · Spinal cord · Trauma

Introduction

Reactive axonal swellings are characteristic features of experimental and human neuropathological processes [10, 22, 36]. Such swellings occur at the severed ends of the proximal and distal stumps of transected axons [10]. Arrested axonal transport is one fundamental mechanism in the production of reactive axonal swellings [10]. The present study concerns accumulation of β-amyloid precursor protein (βAPP) and ubiquitin in axons of the human spinal cord subjected to injury, since both proteins have important biological functions and can serve as markers of axonal injury [42, 44].

βAPP is a glycoprotein [18, 49] which, after synthesis in the perikaryon of neurons, undergoes fast anterograde axonal transport [21, 47]. Many recent immunohistochemical studies have indicated that βAPP accumulates in axons after different types of injuries to the human brain, including trauma [8, 44, 46]. The same phenomenon occurs in experimental brain trauma [34], in ischaemic injuries [17] and in lesions induced by excitotoxins [19, 33]. There are no previous reports on βAPP accumulation after spinal cord trauma in humans, but aggregation of βAPP-immunoreactive material occurs in the rat spinal cord subjected to compression injury [24].

The fate of reactive axonal swellings is not fully known [37]. The traditional view is that the swellings ultimately degenerate but in a well-controlled study on trauma of the cat brain it was shown that, whereas some swellings may degenerate, some may persist unchanged, while others may be the origin of reactive axonal sprouts [37]. Obviously, many degenerative and regenerative changes occur in the reactive axonal swellings.

Degradation of various components in reactive axonal swellings may result from lysosomal and non-lysosomal activities. Mammalian cells have separate systems for lysosomal and non-lysosomal degradation of proteins [4]. Compounds that enter cells from the extracellular environment are usually degraded in lysosomes, whereas non-lysosomal mechanisms are involved in the highly selective removal of intracellular proteins [4].

A great deal of attention has recently been paid to a non-lysosomal proteolytic mechanism which involves the action of ubiquitin [4, 13, 14]. This low molecular mass (8 kDa) protein has very important roles in normal cells

S. Ahlgren (✉) · G. L. Li · Y. Olsson
Laboratory of Neuropathology, University Hospital,
S-751 85 Uppsala, Sweden
Fax: 46-18-55-27-39

Table 1 Survey of the cases with spinal cord trauma

Case no.	File no.	Sex	Age at trauma (years)	Survival period	Cause of trauma
1	134–74	F	49	10 days	Extradural hematoma
2	17–74	M	–	17 days	Complicated breech delivery
3	36–77	M	68	3 weeks	Fall
4	99–82	N	63	1 month	Fall, ankylosing spondylitis
5	284–84	M	32	1 month	Motor-vehicle accident
6	69–84	M	46	14 months	Grand-mal attack
7	295–78	F	< 74	< 2 years	Cervical spondylosis
8	228–76	M	< 46	7 years	Motor-vehicle accident
9	372–84	F	79	6 years	Tumor compression

[4, 6, 9, 13, 14] and is included in the stress protein family [26]. One function of ubiquitin concerns its involvement in proteolytic degradation of abnormal and denatured proteins [4, 13]; ubiquitin can target such proteins for destruction within cells [4, 13]. Ubiquitin is seen in many chronic degenerative central nervous system diseases characterised by the formation of inclusion bodies [7, 15, 26–28, 35]. Ubiquitin immunoreactivity has been visualised in axonal swellings after human brain and spinal cord trauma [29].

Axonal injuries are fundamental changes seen in cases of trauma of the human cord. βAPP and ubiquitin are two proteins which may be implicated in the axonal changes seen after such lesions. Since there have been no previous reports on axonal accumulation of βAPP in human spinal cord trauma and only one investigation regarding expression of ubiquitin [29], the present immunohistochemical investigation was performed to determine whether these two compounds accumulate in axons of human cases with spinal cord injuries.

Material and methods

Post-mortem spinal cord tissue was obtained from six control cases without evidence of trauma and from nine cases with spinal cord injury (Table 1). The cases with trauma ranged in age between 17 days and 79 years and the survival period after injury varied between 10 days to several years. The post-mortem delay was 2–4 days.

Cases with spinal cord trauma

Case 1

A woman, aged 49 years, had a Schwannoma at C4–5 removed 3 years before her death. She was operated on again 20 days before death due to tumour recurrence. After 10 days, she suddenly developed paresis and breathing difficulties, clinically indicating a lesion at C3. She died from anoxic brain injury. Post-mortem examination indicated lobar pneumonia and a pronounced extradural haematoma and necrosis, about 5 cm in length, at C4–5.

Case 2

A boy, 17 days old, died after a complicated breech delivery. He had breathing difficulties and unilateral paresis of the diaphragm. The respiratory insufficiency grew gradually worse, leading to death. On post-mortem examination marked pulmonary atelectasis, old leptomeningeal bleedings around the cerebellum and rupture of the tentorium as well as a fracture of the right humerus were found. The lower cervical cord contained a small area of bleeding in the grey matter and there was a unilateral degeneration of one ventral column.

Case 3

A man aged 68 years with a history of alcohol abuse had fallen in his home. Thereafter, gait disturbance and muscular weakness of the arms appeared. He improved but died from a heart attack 3 weeks later. Post-mortem examination revealed recent myocardial infarction. Macroscopically the spinal cord was normal, but microscopically a lesion in the cervical cord with oedema, slight reactive gliosis and loss of some ventral motor neurons was seen. Below the lesion some degeneration of the lateral and to a minor extent of the ventral columns was evident.

Case 4

A man aged 63 years with ankylosing spondylitis had suffered a minor trauma 1 month before death. He became tetraplegic. Clinical examination indicated a lesion at C6. He died from pronounced respiratory insufficiency. Post-mortem examination indicated marked pulmonary emboli. The vertebra at the level of C6 was fractured. The lower part of the cervical cord was swollen, necrotic and around the cord there was evidence of haemorrhages.

Case 5

A man aged 32 years became tetraplegic below C6 after a motorcycle accident. Radiography showed fractures of vertebrae and bony fragments influencing the cord were removed 6 days later. He died 1 month after the accident. Post-mortem examination revealed large pulmonary emboli. The spinal cord was markedly swollen over a length of about 5 cm (Fig. 1). Microscopy showed severe necrosis particularly of the grey matter. The longitudinal tracts were also injured.

Case 6

A man aged 47 years, mentally retarded with epilepsy, suddenly became tetraplegic in association with a grand mal attack. He died 14 months later. On post-mortem examination there were several old necrotic injuries in the left cerebral hemisphere, presumably caused by birth injuries. The C2–4 region was markedly atrophic, measuring only a few millimetres in thickness. Microscopy revealed an old, severe, necrotic injury.

Fig. 1 Macroscopical view of the spinal cord of case 5. At the site of the injury the tissue is markedly swollen and caudal to the injury there is atrophy of the cord

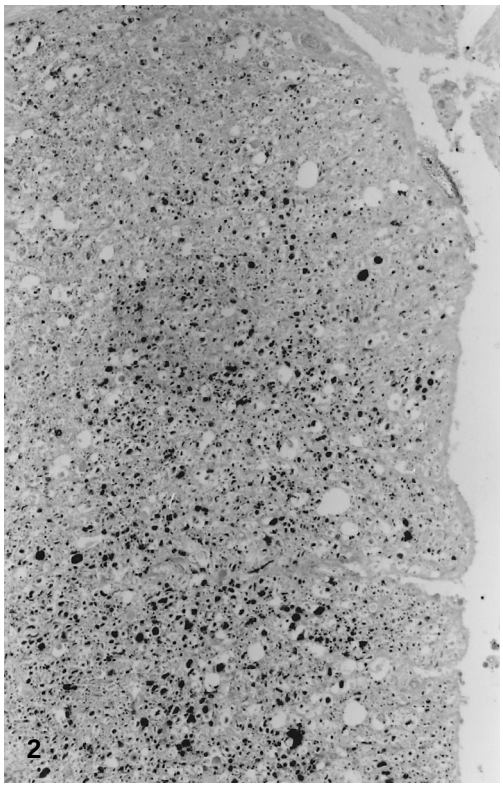

Fig. 2 Transverse section from the spinal cord from case 5 immunostained for β-amyloid precursor protein (βAPP). Sample obtained from one lateral tract. Numerous intensely stained axonal profiles are seen, some of them markedly distended

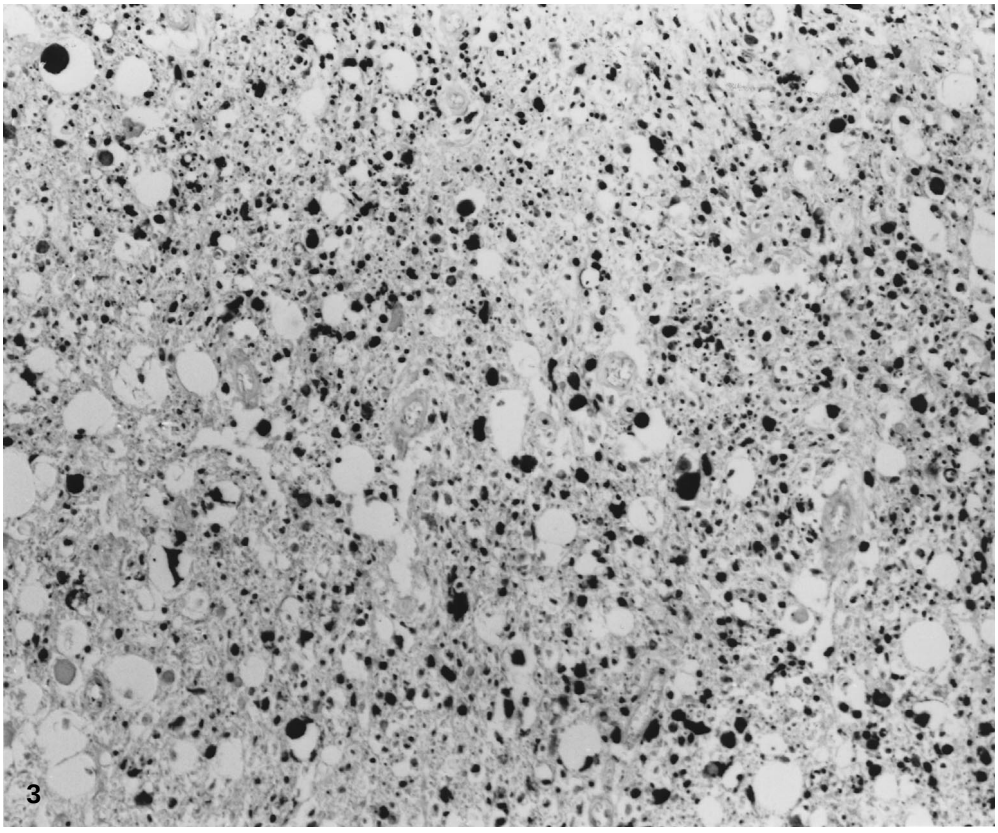

Fig. 3 Higher magnification demonstrating βAPP immunostaining in axons of the same case as in Figs. 1 and 2

Case 7

A woman aged 76 years had been in hospital for the last 2 years with signs of cervical cord compression due to spondylosis. She died from respiratory insufficiency. Post-mortem examination indicated pneumonia and the spinal cord was compressed at C4 by spondylosis. There was a pronounced injury with almost complete destruction of the tissue and marked secondary degeneration in the lateral and ventral columns caudal to the injury.

Case 8

A man aged 49 years with a history of alcohol abuse developed paraplegia after a traffic accident 7 years before death. Post-mortem examination showed that the lower thoracic cord was moderately atrophic over a length of about 6 cm. Ventral motor neurons showed signs of chromatolysis.

Case 9

A woman aged 85 years had, for the last 6 years of her life, signs of an inoperable tumour around the cervical cord. Her legs were paretic and the motility of her hands was reduced. She died from respiratory disturbance. Post-mortem examination indicated an old myocardial infarction. The upper cervical cord was surrounded by an intradural meningioma, 2 cm in length, compressing the cord which was markedly atrophic. The dorsal and ventral columns were moderately degenerated.

Control cases without spinal cord trauma

Case 10

A woman aged 45 years had a history of non-insulin-dependent diabetes mellitus. On post-mortem examination a moderate cardiosclerosis with myocardial infarction and cardiac failure, interstitial nephritis and aspiration pneumonia were found.

Case 11

A man, 53 years old, had suffered from insulin-dependent diabetes mellitus. Post-mortem examination revealed severe cardiosclerosis with several old myocardial infarctions and an old cerebral lesion in the left hemisphere, probably due to a previous stroke.

Case 12

A woman, 60 years old, was suffering from small-cell carcinoma of the lung and chronic obstructive lung disease with respiratory insufficiency. Post-mortem examination demonstrated liver and lymph node metastases and tracheo-bronchitis.

Case 13

A woman aged 79 years with no history of severe disease was found dead at home. Post-mortem examination revealed severe cardiosclerosis, together with an old myocardial infarction and signs of cardiac failure.

Case 14

A man, 84 years old, had suffered from asbestosis and ischaemic heart disease. On post-mortem examination severe cardiosclerosis with myocardial infarction and signs of cardiac failure were found.

Case 15

A man aged 86 years had suffered from schizophrenia. Post-mortem examination revealed severe cardiosclerosis with an old myocardial infarction, pulmonary artery emboli and signs of cardiac failure.

Morphological methods

Portions of the cord, neocortex, hippocampus, cerebellum and pons, fixed in 10% formalin, were taken for examination. Cross-sections were obtained from the spinal cord of all cases. In addition, longitudinal sections from a region immediately below the lesion were examined in cases 4 and 5. The samples were dehydrated, infiltrated with paraffin overnight at 54° C, and embedded in Paraplast. Sections 5 mm thick were placed on slides and the Paraplast was removed. For diagnostic purpose sections were stained by haematoxylin-eosin and the Luxol fast blue method.

Immunostaining

After deparaffination sections were immunostained for β-amyloid, βAPP and ubiquitin. The β-amyloid sections were pretreated with concentrated formic acid for 2 min. The βAPP and ubiquitin sections were treated in a microwave oven in citric buffer for 10 min. Endogenous peroxidase activity was blocked by incubation in 1% H_2O_2 in phosphate-buffered saline (PBS) for 30 min. Sections were then incubated with 20% normal rabbit serum in PBS for 30 min. Monoclonal antibodies against residues 8–17 of β-amyloid (clone 6F/3D; Dakopatts, Glostrup, Denmark) and against the NH_2 domain of the βAPP antigen (clone 22C11; Boehringer Mannheim, Mannheim, Germany) were used at a dilution of 1:100 in 0.1% bovine serum albumin (BSA). In addition, we used an antibody against both free and conjugated ubiquitin (code no. MAB 1510; Chemicon, Temecula, Calif.) at a dilution of 1:20 000 in 0.1% BSA. Incubation was conducted at 4°C overnight.

Antibodies to ubiquitin also reveal ubiquitin cross-reactive protein (UCRP) immunoreactivity [11]. UCRP exhibits significant homology to ubiquitin [11]. This protein, like ubiquitin, is involved in intracellular protein degradation [25]. URCP is expressed in cultured cells from many different tissues after interferon induction [1]. It is not known if this protein is expressed in the central nervous system.

Antibodies bound to antigens were located by the avidin-biotin-peroxidase complex method using 3,3´-diaminobenzidine tetrahydrochloride as chromogen (Vectastain Elite Kit; Vector Laboratories, Burlingame, Calif.). The sections were exposed to biotin-conjugated rabbit anti-mouse IgG and for 30 min at a dilution of 1:200. To intensify the reaction product we applied the nickel enhancement procedure combined with the glucose oxidase method [12, 16, 48].

Results

βAPP immunoreactivity

The spinal cord of the control cases presented βAPP immunoreactivity in nerve cell bodies and a few axonal profiles, but not in dendrites. Occasional subpial astrocytes were faintly stained.

Seven of the nine cases with spinal cord trauma showed an accumulation of βAPP-immunoreactive material in expanded axons of the longitudinal tracts at the site of the injury (Figs. 2, 3; Table 2). Five of the trauma cases presented immunoreactivity in axons of the grey matter; such profiles were more frequent and stained more intensely than those of the controls. Two cases did not show any axonal βAPP immunostaining: case 2, a new-born child with injury as a result of a complicated breech delivery and case 9, an elderly woman with a 6-year history of tumour compression.

Besides labelling of expanded axons, there was a diffuse dotted pattern of βAPP immunoreactivity, resembling dust. Such deposits were present in regions with βAPP-immunoreactive axons and to a lesser extent in other white matter areas. In trauma cases the dust-like βAPP reaction occupied at least one fourth of the white matter

Table 2 Axonal b-amyloid precursor protein (βAPP) and ubiquitin immunostaining in cases with spinal cord trauma (- no stained axons, + few, ++ moderate, +++ many immunoreactive axons)

Case no.	Age at trauma	Survival period	Cause of trauma	βAPP stained axons	Ubiquitin stained axons
1	49	10 days	Extradural haematoma	+	++
2	–	17 days	Complicated breech delivery	–	+
3	68	3 weeks	Fall	++	++
4	63	1 month	Fall, ankylosing spondylitis	+++	++
5	32	1 month	Motor-vehicle accident	+++	++
6	46	14 months	Grand-mal attack	++	+
7	< 74	< 2 years	Cervical spondylosis	+	+
8	< 46	< 3 years	Motor-vehicle accident	+	+
9	79	6 years	Tumor compression	–	+

area. The detailed cellular localisation of this material is unknown. β-Amyloid immunoreactivity was not found in the axons.

Ubiquitin-like immunoreactivity

No ubiquitin-like immunoreactivity was found in axons or in the nerve cell bodies in control cases. In two of the control cases a few ubiquitin-positive glial cells were seen in the white matter.

In cases with trauma a positive ubiquitin-like reaction was found in many of the expanded axons. The number of positive axons was approximately the same as that seen in the βAPP-stained sections (Table 2).

Discussion

The present investigation of focal injuries of the human spinal cord shows that trauma of different types can be associated with deposition of βAPP-immunoreactive material in axons particularly of the longitudinal tracts. Secondly, our study confirms the results of one previous investigation demonstrating ubiquitin-like immunoreactive material in damaged axons of human cases with spinal cord trauma [30].

βAPP is synthesised in the cell body of normal neurons and is carried by fast axonal transport towards the periphery [21, 47]. Focal damage leads to impaired transport and accumulation of many compounds proximal to the injury, as previously demonstrated in human cases with brain trauma [44–46] and in a variety of experimental brain lesions [19, 20, 33, 34] and experimental spinal cord trauma [24]. Our study shows that this phenomenon also occurs in axons of the human spinal cord subjected to a variety of lesions.

There have only been a few studies in which βAPP immunohistochemistry has been applied to human spinal cord: one demonstrated βAPP in dystrophic axons in cases of infantile neuroaxonal dystrophy and ageing [5], and another showed βAPP immunoreactivity in anterior horn cells of the fetal spinal cord [2].

The most marked accumulation of βAPP-immunoreactive material was present in the adult cases with a survival period of about 1 month. Some βAPP-immunoreactive axons were present in the spinal cord of patients with very long survival periods. We do not know if this reflects a long-lasting disturbance of axonal flow or if other mechanisms may account for the abnormality. Most previous experimental investigations on βAPP accumulation in axons have dealt with the early period after trauma and we have not found any long-term studies covering periods of several months after trauma. Such information would be of interest in view of the fact that metabolites of βAPP have important biological functions [43, 49].

Biochemical studies have shown that βAPP can be processed by several proteolytic pathways that generate different breakdown products, e.g. β-amyloid protein, which accumulates in the brain of patients with Alzheimer's disease and cases with severe brain injury [39, 40, 49].A secretory pathway generates soluble non-amyloidogenic amino-terminal derivatives. Such soluble forms have several physiological functions, such as an influence on cell proliferation, regulation of proteases and blood coagulation and involvement in cell adhesion mechanisms [49]. The soluble derivatives may also protect cultured neurons against excitotoxic or ischaemic insults by reducing intracellular calcium ions and modifying calcium responses to glutamate [31, 32]. In conclusion, if soluble forms of βAPP metabolites are formed, they may influence several secondary pathological processes occurring after injury to the cord.

Our study indicates that there is a ubiquitin-like immunoreaction in expanded axons after human spinal cord trauma – a finding in line with those described in an earlier report on human spinal cord injury [23, 30]. Ubiquitin is produced by all eukaryotic cells [38]. Recent studies on ubiquitin mRNA in rat motor neurons indicate that the protein is synthesised in the perikaryon of neurons and then transported into the axon [3, 41]. Ubiquitin has the capacity to identify and bind to abnormal proteins to form complexes that can be degraded by proteolytic enzymes. Ubiquitin is thus one compound which may be of importance in the non-lysosomal degradation of proteins which may occur after an axonal injury.

Acknowledgements This work was supported by grants from the Swedish Medical Research Council project no. 0320, Hedlund's foundation, Åhlén's foundation, Thyring's foundation and O. E. and Edla Johansson's foundation.

References

1. Ahrens PB, Besancon F, Memet S, Ankel H (1990) Tumor necrosis factor enhances induction by β-interferon of a ubiquitin cross-reactive protein. J Gen Virol 71: 1675–1682
2. Arai H, Higuchi S, Matsushita S, Yuzuriha T, Trojanowski JO, Lee VMY (1994) Expression of beta-amyloid precursor protein in the developing human spinal cord. Brain Res 642: 132–136
3. Bizzi A, Schaetzle B, Patton A, Gambetti P, Autillo GL (1991) Axonal transport of the two major components of the ubiquitin system. Free ubiquitin and ubiquitin carboxyl-terminal hydrolase PGP9.5. Brain Res 548: 292–299
4. Ciechanover A (1993) The ubiquitin-mediated proteolytic pathway. Brain Pathol 3: 67–75
5. Cochran E, Bacci B, Chen Y, Patton A, Gambetti P, Autilio-Gambetti L (1991) Amyloid precursor protein and ubiquitin immunoreactivity in dystrophic axons is not unique to Alzheimer's disease. Am J Pathol 139: 485–489
6. Finley D, Varshavsky A (1985) The ubiquitin system: functions and mechanisms. Trends Biochem Sci 10: 343–347
7. Garofalo O, Kennedy PGE, Swash M, Martin JE, Luthert P, Anderton BH, Leigh PN (1991) Ubiquitin and heat shock protein expression in amyotrophic lateral sclerosis. Neuropathol Appl Neurobiol 17: 39–45
8. Gentleman SM, Nash MJ, Sweeting CJ, Graham DI, Roberts GW (1993) β-Amyloid precursor protein (ßAPP) as a marker for axonal injury after head injury. Neurosci Lett 160: 139–144
9. Goldstein G, Sheid M, Hammerling U, Boyse EA, Schlesinger DH, Haill HD (1975) Isolation of a polypeptide that has lymphocyte-differentiating properties and is probably represented universally in living cells. Proc Natl Acad Sci USA 72: 11–15
10. Griffin JW, Price DL, Engel WK, Drachman DB (1977) The pathogenesis of reactive axonal swellings: role of axonal transport. J Neuropathol Exp Neurol 36: 214–227
11. Haas AL, Ahrens P, Bright PM, Ankel H (1987) Interferon induces a 15 kDa protein exhibiting marked homology to ubiqutin. J Biol Chem 262: 11315–11323
12. Hancock MB (1984) Visualization of peptide-immunoreactive processes of serotonin-immunoreactive cells using two color immunoperoxidase staining. J Histochem Cytochem 32: 311–314
13. Hershko A (1991) The ubiquitin pathway for protein degradation. Trends Biochem Sci 16: 265–268
14. Hershko A, Ciechanover A (1986) The ubiquitin pathway for the degeneration of intracellular proteins. Prog Nucleic Acid Res Mol Biol 33: 19–56
15. Ito H, Hirano A (1994) Comparative study of spinal cord ubiquitin expression in post-poliomyelitis and sporadic amyotrophic lateral sclerosis. Acta Neuropathol 87: 425–429
16. Itoh K, Konishi A, Nomura S, Mizuno N, Nakamura Y, Sugimoto T (1979) Application of coupled oxidation reaction to electron microscopic demonstration of horseradish peroxidase: cobalt-glucose oxidase method. Brain Res 175: 341–346
17. Kalaria RN, Bhatti SU, Palatinsky EA, Pennington DH, Shelton ER, Chan HW, Perry G, Lust WD (1993) Accumulation of the β-amyloid precursor protein at sites of ischemic injury in rat brain. NeuroReport 4: 211–214
18. Kang J, Lemaire H, Unterbeck A, Salbaum J, Master C, Grzeschik K, Multhaup G, Beyreuther K, Muller-Hill B (1987) The precursor of Alzheimer's disease amyloid A4 resembles a cell surface receptor. Nature 325: 733–736
19. Kawarabayashi T, Shoji M, Harigaya Y, Yamaguchi H, Hirai S (1991) Expression of APP in the early stage of brain damage. Brain Res 563: 334–338
20. Kawarabayashi T, Shoji M, Yamaguchi H, Tanaka M, Harigaya Y, Ishiguro K, Hirai S (1993) Amyloid β protein precursor accumulates in swollen neurites throughout rat brain with aging. Neurosci Lett 153: 73–76
21. Koo EH, Sisodia SS, Archer DR, Martin LJ, Weidemann A, Beyruther K, Fischer P, Masters CL, Price DL (1990) Precursor of amyloid protein in Alzheimer's disease undergoes fast anterograde axonal transport. Proc Natl Acad Sci USA 87: 1561–1565
22. Lampert P (1967) A comparative electron microscopic study of reactive, degenerating, regenerating and dystrophic axons. J Neuropathol Exp Neurol 26: 345–368
23. Li GL, Farooque M (1996) Expression of ubiquitin-like immunoreactivity in axons after compression trauma to rat spinal cord. Acta Neuropathol 91 : 155–160
24. Li GL, Farooque M, Holtz A, Olsson Y (1995) Changes of beta-amyloid precursor protein after compression trauma to the spinal cord: an experimental study in the rat using immunohistochemistry. J Neurotrauma 12: 269–277
25. Loeb KR, Haas AL (1994) Conjugates of cross- reactive protein distribute in a cytoskeletal pattern. Mol Cell Biol 14: 8408–8419
26. Lowe J, Mayer RJ (1990) Ubiquitin, cell stress and diseases of the nervous system. Neuropathol Appl Neurobiol 16: 281–291
27. Lowe J, Mayer RJ, Landon M (1993) Ubiquitin in neurodegenerative diseases. Brain Pathol 3: 55–65
28. Manetto V, Abdul-Karim FW, Perry G, Tabaton M, Autilio-Gambetti L, Gambetti P (1989) Selective presence of ubiquitin in intracellular inclusions. Am J Pathol 134: 505–513
29. Martin JE, Mather KS, Swash M, Garofalo O, Dale GE, Leigh PN, Anderton BH (1990) Spinal cord trauma in man: studies of phosphorylated neurofilament and ubiquitin expression. Brain 113: 1553–1562
30. Martin JE, Mather KS, Swash M, Garofalo O, Dale GE, Leigh PN, Anderton BH (1990) Spinal cord trauma in man: studies of phosphorylated neurofilament and ubiquitin expression. Brain 113: 1553–1562
31. Mattson M, Barger S, Cheng B, Lieberburg I, Smith-Swintosky V, Rydel R (1993) β-Amyloid precursor protein metabolites and loss of neuronal calcium homeostasis in Alzheimer's disease. Trends Neurosci 16: 409–414
32. Mattson MP, Cheng B, Culwell AR, Esch FS, Lieberburg I, Rydel RE (1993) Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the beta-amyloid precursor protein. Neuron 10: 243–254
33. Nakamura Y, Takeda M, Niigawa H, Hariguchi S, Nishimura T (1992) Amyloid β-protein precursor deposition in rat hippocampus lesioned by ibotenic acid injection. Neurosci Lett 136: 95–98
34. Otsuka N, Tomonaga M, Ikeda K (1991) Rapid appearance of β-amyloid precursor protein immunoreactivity in damaged axons and reactive glial cells in rat brain following needle stab injury. Brain Res 568: 335–338
35. Perry G, Friedman R, Shaw G, Chau V (1987) Ubiquitin is detected in neurofibrillary tangles and senile plaque neurites of Alzheimer disease brains. Proc Natl Acad Sci USA 84: 3033–3036
36. Povlishock JT (1993) Traumatic brain injury: the pathobiology of injury and repair. In: Gorio A (ed) Neuroregeneration. Raven Press, New York, pp 185–217
37. Povlishock JT, Becker DP (1985) Fate of reactive axonal swellings induced by head injury. Lab Invest 52: 540–552
38. Rechsteiner M (1988) Ubiquitin. Plenum Press, New York
39. Roberts GW, Gentleman SM, Lynch A, Graham DI (1991) βA4 amyloid protein deposition in brain after head trauma. Lancet 338: 1422–1423
40. Roberts GW, Gentleman SM, Lynch A, Murray L, Landon M, Graham DI (1994) β-Amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease. J Neurol Neurosurg Psychiatry 57: 419–425
41. Savedia S, Kiernan JA (1994) Increased production of ubiquitin mRNA in motor neurons after axotomy. Neuropathol Exp Neurol 20: 577–586

42. Schweitzer JB, Park MR, Einhaus SL, Robertson JT (1993) Ubiquitin marks the reactive swellings of diffuse axonal injury. Acta Neuropathol 85: 503–507
43. Selkoe DJ (1994) Normal and abnormal biology of the beta-amyloid precursor protein. Annu Rev Neurosci 17: 489–517
44. Sheriff FE, Bridges LR, Gentleman SM, Sivaloganathan S, Wilson S (1994) Markers of axonal injury in post mortem human brain. Acta Neuropathol 88: 433–440
45. Sherriff FE, Bridges LR, Jackson P (1994) Microwave antigen retrieval enhances β-amyloid precursor protein immunoreactivity in formalin-fixed brain in Alzheimer's disease and after head injury. NeuroReport 5: 1085–1088
46. Sherriff FE, Bridges LR, Sivaloganathan S (1994) Early detection of axonal injury after human head trauma using immunocytochemistry for β-amyloid precursor protein. Acta Neuropathol 87: 55–62
47. Shigematsu K, McGeer P (1992) Accumulation of amyloid precursor protein in neurons after intraventricular injection of colchicine. Am J Pathol 140: 787–794
48. Shu S, Ju G, Fan L (1988) The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system. Neurosci Lett 85: 169–171
49. Smith C, Anderton D (1994) The molecular pathology of Alzheimer's disease: are we any closer to understanding the neurodegenerative process? Neuropathol Appl Neurobiol 20: 322–338