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Differential effects of apolipoprotein E isoforms on phosphorylation at specific sites on tau by glycogen synthase kinase-3β identified by nano-electrospray mass spectrometry

Graham M. Gibb[a,1,*], Janice Pearce[a,1], Joanna C. Betts[b], Simon Lovestone[a], Michael M. Hoffmann[c], Winfried Maerz[c], Walter P. Blackstock[b], Brian H. Anderton[a]

[a]*Department of Neuroscience, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, UK*
[b]*Biomolecular Structure Unit, GlaxoWellcome Research and Development, Stevenage SG1 2NY, UK*
[c]*Klinicum der Albert-Ludwigs-Universität, Freiburg, Germany*

Abstract Previously published data have shown an allele-specific variation in the in vitro binding of apolipoprotein E (apoE) to tau, which prompted the hypothesis that apoE binding may protect tau from phosphorylation, apoE3 being more efficient than apoE4. We have, therefore, investigated the effects of apoE on tau phosphorylation in vitro by the proline-directed kinase, glycogen synthase kinase (GSK)-3β. The phosphopeptide maps of tau alone, of tau with apoE3 and of tau with apoE4 were very similar. When apoE2 was present a further four spots were evident. Additionally, of the 15 peptides phosphorylated in the presence or absence of apoE, subtle differences, some isoform-specific, in the relative amounts of phosphorylation were observed. © 2000 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

Key words: Tau; Phosphorylation; Apolipoprotein E; Glycogen synthase kinase-3β; Nano-electrospray mass spectrometry; Two-dimensional phosphopeptide mapping

1. Introduction

The microtubule-associated protein tau is a phosphoprotein predominantly expressed by neurones and contributes to the stability and integrity of neuronal microtubules [1,2]. Regulation of the role of tau in maintaining microtubule stability appears to be largely through phosphorylation [3]. Increased phosphorylation of tau results in a reduction in microtubule binding and assembly competence [4–8] and it is noteworthy that in tangle-bearing neurones in which hyperphosphorylated tau comprises paired helical filaments (PHFs), microtubules are absent [9,10]. To date, 25 phosphorylation sites have been identified by sequence analysis in PHF-tau [11,12] and many of these sites are serine or threonine residues followed in sequence by proline. In vitro studies have shown that several kinases can phosphorylate sites found in PHF-tau (reviewed in [13]) but that in transfected cells overexpressing these kinases it is only glycogen synthase kinase (GSK)-3β and GSK-3α that phosphorylate tau to a comparable extent to that found in vitro [14].

The only genetic variation unequivocally associated with late-onset Alzheimer's disease (AD) is allelic variability at the apolipoprotein E (apoE) locus. Three common polymorphisms of the single apoE gene, alleles ε2, ε3 and ε4, encode isoforms differing at two sites. Gene dosage of apoE ε4 has been shown to be a risk factor for late-onset familial and sporadic forms of AD [15–19]. In vitro binding studies have shown allele-specific variation of interaction between tau and apoE [20]. Whilst both apoE3 and apoE4 are able to bind tau to some degree, apoE3 but not apoE4 is capable of resilient association over a wide range of concentrations [21]. It has been hypothesised, therefore, that apoE may play a protective role, with apoE3 (and apoE2) binding to tau, interfering with its hyperphosphorylation and so preventing or reducing microtubule instability and the formation of PHFs [22]. In this study we have examined the effect of the three common human apoE isoforms on the phosphorylation of tau by GSK-3β by two-dimensional (2-D) phosphopeptide mapping and identified the sites of phosphorylation directly from the 2-D maps using nano-electrospray (nano-ES) mass spectrometry (MS).

2. Materials and methods

2.1. Cloning and expression of human apoE2, E3 and E4 in baculovirus

pAc-E3 (kindly provided by Dr Alan D. Attie, Department of Biochemistry, University of Madison), which is a derivative of pAcYM1 [23], was digested with *Bam*HI to isolate the apoE3 cDNA. This fragment was cloned into the vector pALTER1 (Promega, Madison, WI, USA). Mutagenesis was done according to the manufacturer's instructions. After mutagenesis, the apoE2 and apoE4 cDNA was cloned into the *Bam*HI restriction site of the baculovirus transfer vector pAcYM1. The sequence of the apoE2 and apoE4 cDNA constructs was verified by DNA sequencing prior to cotransfection.

Cotransfection of BaculoGoldTM baculovirus DNA (PharMingen, San Diego, CA, USA) and the transfer vectors for apoE2, apoE3 and apoE4 was performed using *N*-[1-(2,3-dioleoyloxypropyl)]-*N,N,N*-trimethylammonium propane (Boehringer Mannheim, Mannheim, Germany) as transfection reagent. At day 4 after transfection, the supernatant was collected and single virus clones were isolated as described [23]. To produce recombinant apoE on a large scale, 2×10^6 Sf21 cells per ml were seeded in a spinner flask. The cells were infected with a high-titre stock solution of recombinant baculovirus. After incubation

*Corresponding author. Fax: (44)-207-708 0017.
E-mail: g.gibb@iop.kcl.ac.uk

[1] An equal contribution was made by these authors.

Abbreviations: AD, Alzheimer's disease; apoE, apolipoprotein E; BSA, bovine serum albumin; GSK-3β, glycogen synthase kinase-3β; MS, mass spectrometry; nano-ES, nano-electrospray; PHF, paired helical filament

at 27°C for 72–96 h, the apoE-containing supernatant was collected by centrifugation. Recombinant apoE was purified by affinity chromatography on heparin-Sepharose 4B as previously described [24].

2.2. Expression of human apoE in cultured cells

Human apoE3 or apoE4 were expressed in HEK-293 cells stably transfected with either human apoE3 or apoE4 cDNA and kindly supplied by Dr Mary Jo LaDu, University of Chicago [25]. Conditioned medium containing apoE was concentrated approximately 50-fold using Amicon stirred ultrafiltration cells (10 kDa membrane cutoff). The concentrated medium was dialysed overnight at 4°C against phosphate-buffered saline and filter-sterilised before being quantified by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and Coomassie brilliant blue staining against a recombinant apoE standard of known concentration.

2.3. In vitro incubation of tau with apoE and phosphorylation with GSK-3β

Recombinant human tau with one N-terminal insert and four repeat domains (1N4R) was produced in *Escherichia coli* as previously described [26]. Rat GSK-3β was expressed in insect cells using a recombinant baculovirus supplied by J.R. Woodgett (Ontario Cancer Institute, Toronto, Ont., Canada) and purified essentially by the method of Hughes et al. [27], incorporating the modifications of Utton et al. [8]. The reaction conditions described below were used when sites of phosphorylation on tau were to be determined by mass spectrometric sequence analysis from the resulting 2-D phosphopeptide maps. For binding reactions, apoE (20 μg) was incubated with human tau (20 μg) for 1 h at 37°C in 200 μl of 20 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer pH 7.4 (apoE:tau molar ratio of 2:1). Phosphorylation of human tau by GSK-3β was carried out as described [28]. The reaction was terminated by the addition of 0.1 ml of 100% (w/v) trichloroacetic acid and left on ice for 5 min. Precipitated protein was collected by centrifugation at $14\,000 \times g_{av}$ for 5 min and the pellet washed twice with ice-cold 20% (w/v) trichloroacetic acid then twice with ice-cold acetone. Final washed material was resuspended in electrophoresis sample buffer for analysis by SDS–PAGE. In a control set of experiments, tau (2 g) was incubated with bovine serum albumin (BSA) (2, 4 and 10 μg) for 1 h at 37°C in the above buffer prior to phosphorylation.

2.4. Phosphopeptide mapping of tau

Radiolabelled human tau (20 μg) in electrophoresis sample buffer was resolved by 10% (w/v) acrylamide SDS–PAGE, then transferred onto Immobilon P membrane (Millipore) at 15 V for 45 min. Protein was visualised initially by staining the Immobilon P membrane with 0.1% (w/v) amido black solution [29] and radiolabelled protein identified by phosphorimage analysis on a Fujix Bas 1000 imager. The phosphorylated tau bands were excised, counted by Cerenkov radiation and then analysed by 2-D phosphopeptide mapping [28]. Phosphopeptides were visualised by exposing the cellulose plates to phosphorimage analysis and autoradiography at −70°C. Corresponding phosphopeptides were scraped from the two plates, pooled and peptides eluted in 20% (v/v) acetonitrile in water by the method of Affolter et al. [30] for analysis by MS.

2.5. Nano-ES MS

Dried peptides eluted from the cellulose plates were dissolved in 0.5% (v/v) formic acid and desalted using a pulled glass capillary self-packed with ∼5 μl of POROS R3 sorbent (PerSeptive Biosystems, Cambridge, MA, USA), as described by Wilm et al. [31]. The desalted sample was eluted with 5 μl of 50% (v/v) MeOH, 5% (v/v) formic acid into a 500 μl Eppendorf tube, centrifuged ($14\,000 \times g_{av}$ for 1 min) and 1 μl inserted into the spraying needle. Electrospray mass spectra were acquired on an API III triple quadrupole machine (PE-Sciex, Ont., Canada) equipped with a nano-ES ion source developed by Wilm and Mann [32,33] as described previously [12,34].

2.6. Statistical analysis

For quantitative studies, densitometric analysis of peptide spots resolved by 2-D electrophoresis was performed. Single spots on scanned autoradiographs or phosphorimages of the 2-D maps were identified and, using MetaMorph image analysis software, optical density of each spot determined by thresholding using the same criteria in each case. Five samples from separate experiments for tau

phosphorylated by GSK-3β alone and tau phosphorylated in the presence of apoE2, apoE3 or apoE4 and two from tau phosphorylated in the presence of BSA were measured. The total density for each spot was calculated as a percentage of the sum of the density of all measured spots in each chromatogram (the unresolved mass of aggregated spots close to the origin was not analysed). The resulting mean relative values for each spot for the five separate samples for each experiment were compared using ANOVA with Bonferroni correction for multiple testing using the SPSS statistical package.

3. Results

3.1. Phosphopeptide mapping analysis of recombinant human tau

Recombinant human tau of the isoform containing one amino-terminal region and four microtubule binding domains (1N4R) phosphorylated with GSK-3β in the presence of [γ-³²P]ATP was digested with trypsin and the radiolabelled phosphopeptides separated by 2-D electrophoresis and thin layer chromatography. Autoradiographic analysis of the resulting peptide map revealed 15 consistently well-resolved radiolabelled phosphopeptide spots. A representative example of the phosphopeptide maps is illustrated in Fig. 1. In most experiments, some radiolabelled material, which may represent incompletely digested peptides, remained close to the origin. Resolved spots were individually scraped from two thin layer cellulose plates, and then pooled for extraction of the constituent peptide(s).

3.2. Identification of phosphopeptides and sites of phosphorylation

Phosphopeptides which were extracted from the 2-D phosphopeptide maps of GSK-3β-phosphorylated human tau (1N4R) (Fig. 1) were identified using nano-ES MS. Of the 15 spots, we were unable to identify four phosphopeptide spots (spots 1, 7, 8, 13). Spots 3 and 12 each contained two different phosphopeptides species, numbered 3a and 3b and 12a and 12b, respectively (Table 1).

A total of 12 sites phosphorylated by GSK-3β were identified (Table 1). The GSK-3β phosphorylation sites found in human tau are located in two clusters on either side of the microtubule binding domains, residues 181–235 and 396–404. There is one exception to this; serine 262 which is located in the first microtubule binding domain (Table 1). The GSK-3β-
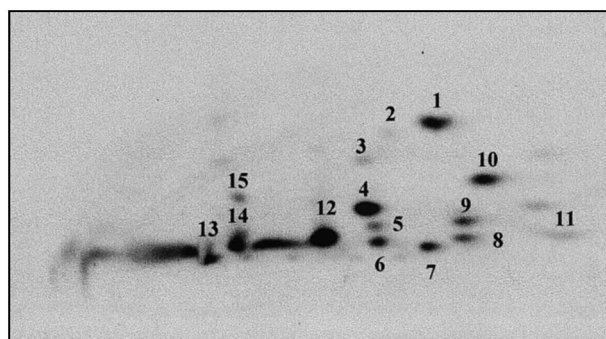

Fig. 1. Phosphopeptide map analysis of tau phosphorylated by GSK-3β. 20 μg of recombinant human tau (1N4R) was incubated with GSK-3β at 30°C for ∼18 h in the presence of [γ-³²P]ATP and then analysed by SDS–PAGE and phosphopeptide mapping as described in Section 2. The 15 numbered phosphopeptide spots which were well-resolved were individually scraped from the plate, eluted and analysed by MS.
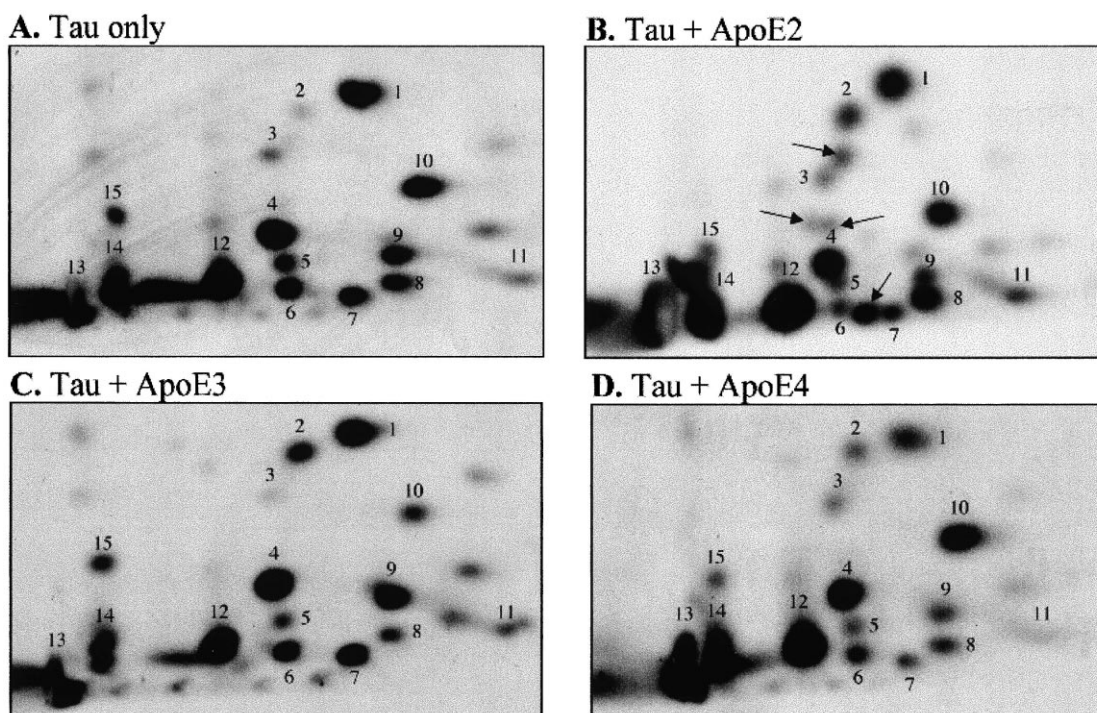
Fig. 2. Phosphopeptide map analysis of recombinant human tau 1N4R phosphorylated by GSK-3β in the absence of any apoE or in the presence of apoE2, apoE3 or apoE4. Tau was incubated in the absence or presence of apoE2, E3 or E4 for 1 h at 37°C, prior to phosphorylation by GSK-3β, SDS–PAGE and 2-D phosphopeptide mapping analysis were as described in the legend to Fig. 1. In the presence of apoE2 (B), several new phosphopeptides (arrows) are found that are absent from the control (i.e. no apoE – A) and from phosphorylation in the presence of apoE3 (C) or apoE4 (D).

specific inhibitor lithium markedly reduces phosphorylation at all the above sites when included in the phosphorylation reaction (data not shown).

3.3. The effects of apoE on tau phosphorylation

Having established reproducible phosphopeptide maps of GSK-3β-phosphorylated human tau isoform 1N4R and identified a majority of these sites, the effects of apoE on tau phosphorylation were investigated. Given previously reported differences in apoE binding apparently being dependent upon the source of the protein [35], we have used two sources of apoE in our studies; firstly, baculoviral expressed recombinant apoE and, secondly, apoE from tissue culture medium

of cells stably expressing different human apoE isoforms. The results did not differ with apoE source and for all quantitative analysis baculoviral expressed recombinant apoE was used. Prior to phosphorylation by GSK-3β, tau was incubated with apoE2, E3, E4 or BSA as a control for 1 h at 37°C.

Phosphopeptide maps of tau pre-incubated with recombinant apoE2, apoE3 or apoE4 prior to phosphorylation by GSK-3β were examined (Fig. 2). The autoradiographs shown in Fig. 2 are representative of several experiments. The pattern of phosphopeptide separation shown by the maps was broadly similar to tau phosphorylated in the absence of apoE, with the usual 15 resolved phosphopeptide spots. However, four additional spots were resolved on the maps when apoE2 was

Table 1
Phosphopeptides identified by nano-ES MS of peptides recovered from 2-D peptide maps of GSK-3β-phosphorylated recombinant human tau (1N4R)

Spot	Residues	Sequence	Average mass (Da)	Site(s) identified
2	260–267	IGSpTENLK	940.9	262
3a	212–221	TpPSLPTPPTR	1146.2	212
3b	212–221	TPSLPTpPPTR	1146.2	217
4	210–221	SRTpPSLPTpPPTR	1469.4	212, 217
5	181–190	TpPPSSGEPPK	1076.1	181
6	231–240	TpPPKSpPSSAK	1159.1	231, 235
9	226–240	VAVVRTpPPKSPSSAK(+1P)	1683.8	231, (235, 237 or 238)
10	210–221	SRTpPSLPTPPTR	1389.5	212
11	231–240	TpPPKSPSSAK	1079.1	231
12a	386–406	TDHGAEIVYKSpPVVSpGDTSpPR	2455.3	396, 400, 404
12b	191–209	SGDRSpGYSSpPGSPGTPGSR	1968.8	195, 199
14	191–209	SGDRSpGYSSPGSPGTpPGSR	2048.8	195, 205 (198, 199 OR 202)
15	212–221	TpPSLPTpPPTR	1226.2	212, 217

Numbering according to the largest tau isoform. Sp and Tp represent phosphorylated serine or threonine residues. Spots 3 and 12 both contained two distinct phosphopeptide species numbered 3a and 3b and 12a and 12b, respectively.
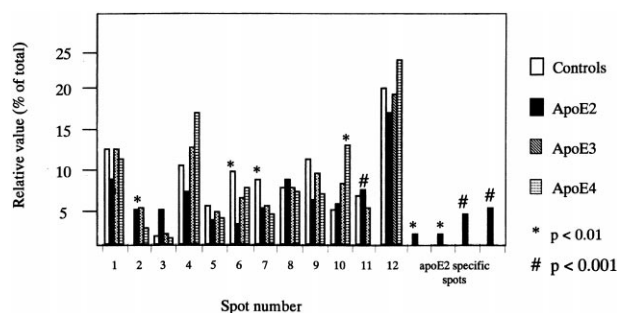
Fig. 3. Densitometric analysis of the phosphopeptide maps showing the effects of apoE isoform on tau phosphorylation by GSK-3β. The relative intensity of each phosphopeptide spot (relative to the summed intensity) averaged in each case from five experiments. * indicates significant difference < 0.01 between apoE isoforms and controls.

present (Fig. 2B) although, perhaps because of the small amount of peptide in the spots, we have been unable to identify these phosphopeptides by mass spectrometric sequencing.

A quantitative analysis of the relative intensity of phosphorylation at the different sites as represented by spot intensities (relative to the total) revealed differences between the absence and presence of apoE, and between apoE isoforms (Fig. 3). The addition of apoE resulted in a decrease in relative phosphorylation of the peptide containing Thr231/Ser235 (spot 6) and an, as yet, unidentified phosphopeptide (spot 7) and an increase in phosphorylation at Ser262 (spot 2) and a peptide containing Thr212 (spot 10).

Of the isoform differences, it was apparent that apoE4 resulted in a significantly larger increase in phosphorylation at the Thr212-containing peptide (spot 10) than the other two apoE isoforms and a markedly reduced phosphorylation at a Thr231-containing peptide (spot 11) when compared with apoE2 or apoE3. Additionally, apoE4 did not reduce phosphorylation at the peptide containing both Thr231 and Ser235 (spot 6) to the same degree as either apoE2 or apoE3.

ApoE2 induced phosphorylation at four novel sites (Fig. 2B, arrows) when compared with tau alone or tau treated with either apoE3 or apoE4. Relative to apoE3 or apoE4, apoE2 significantly increased the intensity of the spot (spot 3) marking the presence of a mixture of small peptides containing either phosphorylated Thr212 or Thr217. Interestingly, this appears to be at the expense of the larger peptide containing both sites (spot 4) which was reduced by apoE2 although the magnitude of the effect just failed to reach statistical significance. The resolution of spots 13–15 from one another was not reliable enough for the above analysis to be carried out. In addition, spot 11 in the apoE4-treated sample gave an average value close to zero.

In order to control for possible non-specific effects of the presence of additional proteins on phosphorylation, phosphorylation was also conducted in the presence of BSA (molar ratio tau:BSA of 1:1, 1:2 and 1:5). There was no significant difference between the maps produced by GSK-3β phosphorylation of tau alone and tau with BSA, either in the pattern or intensity of spots (data not shown).

4. Discussion

The hypothesis that apoE isoforms influence tau phosphor-

ylation derives from previous in vitro studies of isoform-specific interactions of apoE with recombinant tau demonstrating an avid binding with apoE3 but not apoE4, that did not dissociate after electrophoresis on SDS gels run under non-reducing conditions [20]. Here we demonstrate in the presence of reducing agents an effect of apoE isoforms on tau phosphorylation, as hypothesised [36], although the influence of the different isoforms is complex. Firstly, some novel spots were generated in the presence of apoE2 and secondly, a change in the relative intensity in some spots was induced by different apoE isoforms. The methods employed enabled us to compare relative levels of phosphorylation at particular sites with high sensitivity and accuracy.

In light of the recent discovery that recombinant GSK-3β does not phosphorylate Ser262 [37], it is a surprise that our baculovirus-derived GSK-3β kinase preparation does partially phosphorylate this residue in vitro. However we found that the GSK-3β-specific inhibitor, lithium, markedly reduces phosphorylation at this site. In fact Ser262 phosphorylation was increased in the presence of all three isoforms of apoE, possibly due to a conformational change resulting from the association of apoE with tau. There were isoform-specific variations in phosphorylation at other sites. Notably, the Thr231/Ser235 site was more protected by apoE2 than apoE4. Thr212 was significantly more phosphorylated when apoE4 was present than when tau was either alone or bound to apoE2 or apoE3, whereas Thr231 was less phosphorylated when apoE4 was present. It has been suggested that simultaneous phosphorylation of multiple sites may be required for the disruption of microtubule binding by tau and that double phosphorylation at Thr212/Ser214 and Thr231/Ser235 may be unique to PHF-tau [38]. Other results have indicated that PHF-tau results from sequential phosphorylation that includes phosphorylation of Thr212 by GSK-3β followed by Ser214 by PKA. If Ser214 is phosphorylated first, Thr212 is thereby protected [39]. The modest, but consistent and statistically significant isoform-specific effects shown here, in particular the increased phosphorylation of Thr212 in the presence of apoE4 and the failure to protect the Thr231/Ser235 peptide from phosphorylation may, therefore, have an effect by skewing the sequence of phosphorylation that leads to PHF-tau. Similarly, the additional phosphopeptides seen when apoE2 was present may represent sites which, readily phosphorylated by GSK-3β, thereby prevent further phosphorylation by another kinase.

These results provide further evidence that GSK-3β is an important, although not the only tau-kinase and provide further experimental evidence to support the hypothesis that apoE influences tau phosphorylation. Indeed in a recent study in which human apoE4 was overexpressed in transgenic mice [40], it was shown that apoE4 expression in neurones resulted in an increase in phosphorylation of tau protein. Further studies are planned to examine, using nano-ES MS, the phosphorylation of tau in neurones, to determine the significance of any tau–apoE interaction in vivo.

References

[1] Matus, A. (1994) Trends Neurosci. 17, 19–22.
[2] Tucker, R.P. (1990) Brain Res. Rev. 15, 101–120.
[3] Smith, C. and Anderton, B.H. (1994) Neuropathol. Appl. Neurobiol. 20, 328–332.
[4] Bramblett, G.T., Trojanowski, J.Q. and Lee, V.M. (1992) Lab. Invest. 66, 212–222.
[5] Gustke, N., Steiner, B., Mandelkow, E.M., Biernat, J., Meyer, H.E., Goedert, M. and Mandelkow, E. (1992) FEBS Lett. 307, 1999.
[6] Lu, Q. and Wood, J.G. (1993) J. Neurosci. 13, 508–515.
[7] Lovestone, S., Hartley, C.L., Pearce, J. and Anderton, B.H. (1996) Neuroscience 73, 1145–1157.
[8] Utton, M.A., Vandecandelaere, A., Wagner, U., Reynolds, C.H., Gibb, G.M., Miller, C.C.J., Bayley, P.M. and Anderton, B.H. (1997) Biochem. J. 323, 741–747.
[9] Gray, E.G., Paula, B.M. and Roher, A. (1987) Neuropathol. Appl. Neurobiol. 13, 91–110.
[10] Flament, D.J. and Couck, A.M. (1979) Acta Neuropathol. Berl. 46, 159–162.
[11] Morishima, K.M., Hasegawa, M., Takio, K., Suzuki, M., Yoshida, H., Titani, K. and Ihara, Y. (1995) J. Biol. Chem. 270, 823–829.
[12] Hanger, D.P., Betts, J.C., Lovigny, T.L.F., Blackstock, W.P. and Anderton, B.H. (1998) J. Neurochem. 71, 2465–2476.
[13] Lovestone, S. and Reynolds, C.H. (1997) Neuroscience 78, 309–324.
[14] Lovestone, S., Reynolds, C.H., Latimer, D., Davis, D.R., Anderton, B.H., Gallo, J.M., Hanger, D., Mulot, S., Marquardt, B., Stabel, S., Woodgett, J.R. and Miller, C.C.J. (1994) Curr. Biol. 4, 1077–1086.
[15] Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P., Small, G.W., Roses, A.D., Haines, J.L. and Pericak Vance, M.A. (1993) Science 261, 921–923.
[16] Strittmatter, W.J., Saunders, A.M., Schmechel, D., Pericak Vance, M., Enghild, J., Salvesen, G.S. and Roses, A.D. (1993) Proc. Natl. Acad. Sci. USA 90, 1977–1981.
[17] Saunders, A.M., Strittmatter, W.J., Schmechel, D., George Hyslop, P.H., Pericak Vance, M.A., Joo, S.H., Rosi, B.L., Gusella, J.F., Crapper-MacLachlan, D.R., Alberts, M.J., Hulette, C., Crain, B., Goldgaber, D. and Roses, A.D. (1993) Neurology 43, 1467–1472.
[18] Poirier, J., Davignon, J., Bouthillier, D., Kogan, S., Bertrand, P. and Gauthier, S. (1993) Lancet 342, 697–699.
[19] Yu, C.E., Payami, H., Olson, J.M., Boehnke, M., Wijsman, E.M., Orr, H.T., Kukull, W., Goddard, K.A., Nemens, E., White, J.A., Alonso, M.E., Taylor, T.D., Ball, M.J., Kaye, J., Morris, J., Chui, H., Sadovnick, A.D., Martin, G.M., Larson, E.B., Heston, L.L., Bird, T.D. and Schellenberg, G.D. (1994) Am. J. Hum. Genet. 54, 631–642.
[20] Strittmatter, W.J., Saunders, A.M., Goedert, M., Weisgraber, K.H., Dong, L.M., Jakes, R., Huang, D.Y., Pericak Vance, M., Schmechel, D. and Roses, A.D. (1994) Proc. Natl. Acad. Sci. USA 91, 11183–11186.
[21] Fleming, L.M., Weisgraber, K.H., Strittmatter, W.J., Troncoso, J.C. and Johnson, G. (1996) Exp. Neurol. 138, 252–260.
[22] Strittmatter, W.J., Weisgraber, K.H., Goedert, M., Saunders, A.M., Huang, D., Corder, E.H., Dong, L.M., Jakes, R., Alberts, M.J., Gilbert, J.R., Han, S.H., Hulette, C., Einstein, G., Schechmel, D.E., Pericak-Vance, M.A. and Roses, A.D. (1994) Exp. Neurol. 125, 163–171.
[23] Gretch, D.G., Sturley, S.L., Friesen, P.D., Beckage, N.E. and Attie, A.D. (1991) Proc. Natl. Acad. Sci. USA 88, 8530–8533.
[24] Marz, W., Hoffmann, M.M., Scharnagl, H., Fisher, E., Chen, M., Nauck, M.S., Feussner, G. and Wieland, H. (1998) J. Lipid Res. 39, 658–669.
[25] LaDu, M.J., Falduto, M.T., Manelli, A.M., Reardon, C.A., Getz, G.S. and Frail, D.E. (1994) J. Biol. Chem. 269, 23403–23406.
[26] Mulot, S.F., Hughes, K., Woodgett, J.R., Anderton, B.H. and Hanger, D.P. (1994) FEBS Lett. 349, 359–364.
[27] Hughes, K., Pulveret, B.J., Theocharous, P. and Woodgett, J.R. (1992) Eur. J. Biochem. 203, 305–311.
[28] Reynolds, C.H., Gibb, G.M. and Lovestone, S. (1999) Tau phosphorylation both in vitro and in cells, in: Alzheimer's Disease–Methods and Protocols (Hooper, N.M., Ed.), 1st edn., pp. 375–394, Humana Press, NJ.
[29] Gershoni, J.M. and Palade, G.E. (1982) Anal. Biochem. 124, 396–405.
[30] Affolter, M., Watts, J.D., Krebs, D.L. and Aebersold, R. (1994) Anal. Biochem. 223, 74–81.
[31] Wilm, M., Neubauer, G. and Mann, M. (1996) Anal. Chem. 68, 527–533.
[32] Wilm, M. and Mann, M. (1996) Anal. Chem. 68, 1–8.
[33] Mann, M. and Wilm, M. (1994) Anal. Chem. 66, 4390–4399.
[34] Cleverley, K.E., Betts, J.C., Blackstock, W.P., Gallo, J.M. and Anderton, B.H. (1998) Biochemistry 37, 3917–3930.
[35] LaDu, M.J., Pederson, T.M., Frail, D.E., Reardon, C.A., Getz, G.S. and Falduto, M.T. (1995) J. Biol. Chem. 270, 9039–9042.
[36] Benzing, W.C. and Mufson, E.J. (1995) Exp. Neurol. 132, 162–171.
[37] Godeman, R., Biernat, J., Mandelkow, E.M. and Mandelkow, E. (1999) FEBS Lett. 454, 157–164.
[38] Hoffmann, R., Lee, V.M.Y., Leight, S., Vargar, I. and Otvos, L. (1997) Biochemistry 36, 8114–8124.
[39] Zheng, F.Q., Biernat, J., Mandelkow, E.M., Illenberger, S., Godemann, R. and Mandelkow, E. (1998) Eur. J. Biochem. 252, 542–552.
[40] Tesseur, I., Dorpe, J.V., Spittaels, K., Van den Haute, C., Moechars, D. and Van Leuven, F. (2000) Am. J. Pathol. 156, 951–964.