Bradykinin-induced amyloid precursor protein secretion: a protein kinase C-independent mechanism that is not altered in fibroblasts from patients with sporadic Alzheimer's disease

Marco RACCHI*†¹, Paola IANNA†, Giuliano BINETTI*, Marco TRABUCCHI* and Stefano GOVONI‡

*Laboratory of Cellular and Molecular Neurobiology, Alzheimer's Disease Unit, I.R.C.C.S. San Giovanni di Dio, Sacred Heart Hospital-FBF, Brescia, Italy, †Institute of Pharmacology, University of Milano, Milano, Italy, and ‡Institute of Pharmacology, University of Pavia, Pavia, Italy

We treated human skin fibroblasts with bradykinin (BK) and observed a concentration-dependent increase in the release of soluble amyloid precursor protein (sAPP). The estimated EC₅₀ for the observed effect is 2.8 nM, which is of the same order of magnitude as the reported K_d of BK binding in human skin fibroblasts. The effect of BK on sAPP secretion appears to be dependent on interaction of the ligand with the B₂ type of BK receptors but independent of activation of protein kinase C. We also show that sAPP release after BK treatment in fibroblasts from patients with sporadic Alzheimer's disease is not different

from control cells and is paralleled by equivalent levels of inositol trisphosphate production. A discussion of the differences from previously published work focuses on the possible divergent alterations in transduction systems in fibroblasts from patients with familial and sporadic Alzheimer's disease. Our results are the first example of receptor-mediated sAPP release in human skin fibroblasts and the first demonstration of the co-existence of protein kinase C-dependent and -independent mechanisms in these cells.

INTRODUCTION

A hallmark of Alzheimer's disease (AD) is deposition in the brain of β -amyloid protein (A β), a self-aggregating peptide of 39–42 amino acids which is part of a ubiquitous precursor protein [1,2] named amyloid precursor protein (APP). APP follows the normal constitutive secretory pathway and is cleaved by ' α secretase', releasing the ectodomain into the extracellular space [3,4]. Proteolytic processing of APP by α secretase occurs within the sequence of A β , thus precluding its formation [5]. In contrast, processing of APP by β and γ secretases at the N- and C-termini respectively of A β is followed by rapid secretion of the amyloidogenic peptide [6,7].

An unusual cellular mechanism coupled to a variety of extra- and intra-cellular signals regulates the rate at which the precursor is cleaved by α secretase and released as soluble protein into the extracellular space (for a review see ref. [8]). Direct activation of protein kinase C (PKC) by phorbol esters produces an increase in soluble APP (sAPP) released by cells into the culture medium [9–11]. Furthermore, the secretory processing of APP can be accelerated by stimulation of G-protein-coupled receptors, including muscarinic acetylcholine receptors [12–14], serotoninergic receptors [15] and bradykinin (BK) receptors [16].

The rate of formation of A β appears to be inversely related to the release of sAPP. In several cellular systems, activation of intracellular pathways leading to sAPP secretion is paralleled by a reduction in the release of A β [17–19], suggesting that the stimulation of APP processing and the release of sAPP is associated with reduced formation of amyloidogenic derivatives.

These data also suggest that signal-transduction systems that link G-protein-coupled receptors to sAPP release play a major physiopathological role in the regulation of APP metabolism.

Alterations in transduction systems may be a key feature of AD [20] both at brain level and in peripheral tissues such as fibroblasts (for a review see [21,22]). Data from several laboratories have demonstrated a decrease in PKC activity in fibroblasts in sporadic AD [23–25]. Other studies have demonstrated a reduction in β -adrenergic-stimulated cAMP increase linked to abnormal coupling of G-proteins to the β -adrenergic receptor [26]. Finally changes in inositol 1,4,5-trisphosphate (IP₃) production in response to BK have been reported. Elevated IP₃ production in response to BK in fibroblasts from patients with AD correlated positively with an increase in BK receptor number [27]. We have previously demonstrated that alterations in PKC activity directly correlate with alterations in APP metabolism in fibroblasts derived from patients with sporadic AD [28].

Our goal in this paper was to characterize the response of human skin fibroblasts to BK treatment in terms of sAPP release and to assess whether the reported alteration in BK-mediated signal transduction would be reflected in any alteration in APP metabolism in fibroblasts from patients with sporadic AD.

EXPERIMENTAL

Materials

All culture media and supplements were obtained from Gibco Life Technologies (Paisley, Scotland, U.K.). Fetal calf serum was

Abbreviations used: APP, amyloid precursor protein; sAPP, soluble amyloid precursor protein; A β , amyloid protein; PKC, protein kinase C; PdBu, phorbol 12,13-dibutyrate; AD, Alzheimer's disease; BK, bradykinin; B₂ receptor, kinin receptor subtype 2; HOE140, D-Arg [Hyp³, Thi⁵, D-Tic⁷, Oic⁸]BK, where Hyp is hydroxyproline, Thi is L-3-(2-thenyl)alanine, Tic is D-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid and Oic is (3S,7S) (3a,7a)-octahydroindol-2-carboxylic acid; IP₃, inositol 1,4,5-trisphosphate; PS1, presenilin 1; MMSE, Mini-Mental State Examination.

¹ To whom correspondence should be addressed, at: IRCCS 'Centro San Giovanni di Dio - Fatebenefratelli', Via Pilastroni 4, 25123 Brescia, Italy.

purchased from Mascia Brunelli Biolife (Milano, Italy). Electrophoresis reagents were obtained from Bio-Rad (Hercules, CA, U.S.A.). HOE 140 was a gift from Dr. W. Mueller-Esterl, Institute for Physiology, Chemistry and Pathobiochemistry, Johannes Gutenberg University, Mainz, Germany. All other reagents were of the highest grade available and were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) unless otherwise specified.

Patients

Patients included in this study were evaluated in the Alzheimer's Disease Unit of the IRCCS 'Centro San Giovanni di Dio - Fatebenefratelli', Brescia, Italy. The protocol was approved by the local ethical committee. Written consent was obtained from all subjects or, where appropriate, their care providers.

Ten patients with probable AD (seven women and three men) were included in the study. All patients met NINCDS-ADRDA [29] criteria for probable AD; they did not have a familial history of dementia, and were therefore classified as 'sporadic AD' cases. All patients presented a 3–7-year history of progressive cognitive impairment predominantly affecting memory (mean $\pm$ S.D. duration of illness 54.0 ± 14.2 months). Cognitive status was quantified using the Mini-Mental State Examination (MMSE) [30]; the mean $\pm$ S.D. score for AD patients was 9.2 ± 7.4 ($n = 10$).

Thirteen subjects were selected for the non-AD control group (eight women and five men). Among these were included patients with neurological and psychiatric disorders such as slow progressive aphasia (two), paranoid syndrome (one) and normotensive hydrocephalus (one). The mean MMSE value for the non-AD control group was 24.5 ± 8.2 ($n = 13$). In addition, four young healthy volunteers (mean $\pm$ S.D. age 31.2 ± 2.4 years) were selected.

The mean age of the non-AD patients (67.6 ± 6.1 years) was not statistically different from that of the AD patients (70.3 ± 8.6 years).

The consumption of neuroleptics, anti-depressants, benzodiazepines, oral anti-diabetic agents, digitalis and anti-thrombotics was similar in the two groups.

Skin fibroblast cultures

The methodology for obtaining the biopsy specimen of shoulder skin (epidermis plus dermis), establishment of fibroblast cell lines, and growth and storage conditions have been described elsewhere [25]. In accordance with a previous report [31], cell growth was generally comparable in AD and non-AD fibroblasts.

Fibroblast cell lines from AD, non-AD and young control subjects were thawed at the same passage and cultured in Eagle's minimum essential medium supplemented with 10% fetal calf serum, penicillin/streptomycin, non-essential amino acids and Tricine buffer (20 mM, pH 7.4) at 37 °C in 5% CO₂/95% air.

Experimental treatments

Confluent monolayers of cells were washed twice with PBS and once with serum-free culture medium before the different treatments, except where specifically indicated. Incubation was continued for 2 h at 37 °C. BK, [Hyp³]BK and HOE140 were prepared as stock solutions (1 mM) in PBS; staurosporine and phorbol 12,13-dibutyrate (PdBu) were dissolved in DMSO. Controls receiving vehicle alone were included in all experiments. In each experiment in which AD and non-AD patients were compared, the mean age of the two groups and the number of passages of the cells were not statistically different.

Harvesting of the cells and preparation of conditioned medium

Conditioned medium was collected after 2 h of incubation and centrifuged at 13000 *g* for 5 min to remove detached cells. Proteins in the conditioned medium were quantitatively precipitated by the deoxycholate/trichloroacetic acid procedure as described previously [28]. Cell monolayers were washed twice with ice-cold PBS and lysed on the tissue culture dish by addition of ice-cold lysis buffer (50 mM Tris/HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA and 1% Triton X-100) and scraped with a rubber spatula. An aliquot of the cell lysate was used for protein analysis with the Bio-Rad Bradford kit for protein quantification.

Immunodetection of sAPP

Normalization of protein loading on each blot was obtained by loading an equal volume of samples of conditioned medium standardized to total cell lysate protein concentration. Proteins were subjected to SDS/PAGE (10% gel) and then transferred to nitrocellulose membrane (Costar, Cambridge, MA, U.S.A.). For the detection of secreted APP, the monoclonal antibody 22C11 (Boehringer-Mannheim) was used, and the blots were incubated overnight at room temperature. Detection was carried out by incubation with horseradish peroxidase-conjugated goat anti-mouse IgG (Kirkegaard and Perry Labs, Gaithersburg, MD, U.S.A.) for 1 h. The blots were then washed extensively, and sAPP was visualized using an enhanced chemiluminescence method (Amersham). The immunoreactive band detected by antibody 22C11 was also immunoprecipitated by antiserum ER3 β 1-16 (not shown), which recognizes epitopes in the first 16 amino acids of A β (M. Racchi, unpublished work), which also constitute the C-terminus of α secretase-cleaved APP; therefore the identified band can be assumed to be the α secretase-cleaved form of sAPP.

Determination of IP₃ levels

Cells were seeded at a density of 6×10^5 /100 mm Petri dish and incubated under the culture conditions previously described for 5 days. Cells were then washed twice with PBS and incubated with 4 ml of PBS containing 1 μ M BK for 10 s at 37 °C. IP₃ levels were measured using a NEN-DuPont kit (Inositol-1,4,5-Trisphosphate [³H] Radioreceptor Assay Kit).

Densitometry and statistics

Analysis of Western blots was performed by calculating the relative density of the immunoreactive bands after acquisition of the blot image through using a Nikon CCD videocamera module and analysis by means of the Image 1.47 program (Wayne Rasband, NIH, Research Service Branch, NIMH, Bethesda, MD, U.S.A.). The relative densities of the bands were expressed as arbitrary units and normalized to data obtained from control samples run under the same conditions. Controls were processed in parallel with stimulated samples and always included in the same blot. Preliminary experiments with serial dilutions of secreted proteins allowed determination of the optimal linear range of the chemiluminescence reaction. Data from the AD and control groups were compared by one-way analysis of variance followed, when significant, by two-tailed Student's *t* test; *P* < 0.05 was considered significant.

RESULTS

The first objective of our investigation was the characterization of the response of human skin fibroblasts to BK stimulation in terms of sAPP secretion. Cells from four healthy young volunteers

Figure 1 Dose-dependent effect of BK on sAPP release from control fibroblasts

Cells growing in 100 mm Petri dishes were incubated in serum-free medium for 2 h at 37 °C with increasing concentrations of BK ranging from 0.3 nM to 10 μM. (A) Western-blot analysis of sAPP secreted into the conditioned medium after BK treatment. (B) Densitometric analysis of Western-blot immunoreactive bands. Data are expressed as percentages of maximum APP released: each point represents the mean $\pm$ S.E.M. of data obtained from at least three different cell lines.

Table 1 BK-induced sAPP release is dependent on BK B2 receptors and independent of PKC activation

BK (3 nM) and [Hyp³]BK (10 nM) both to induce sAPP release in a quantitatively similar manner. Involvement of the B2 receptor is proven by the inhibition of BK (3 nM)-induced sAPP release by HOE140 (300 nM). Staurosporine (100 nM) does not inhibit BK (3 nM)-induced sAPP release but effectively antagonizes PdBu (300 nM)-mediated responses. Data are expressed as percentages of the basal sAPP release (vehicle treatment) (mean $\pm$ S.E.M. of data obtained from at least three different cell lines). * $P < 0.05$, ** $P < 0.01$, compared with basal sAPP release by Student's *t* test.

Treatment	sAPP release (% of basal)
Vehicle	100.0 $\pm$ 5.6
BK (3 nM)	141.0 $\pm$ 5.4*
[Hyp ³]BK (10 nM)	163.7 $\pm$ 8.6*
HOE140 (300 nM)	112.3 $\pm$ 10.8
BK (3 nM) + HOE 140 (300 nM)	76.3 $\pm$ 16.3
BK (3 nM) + staurosporine (100 nM)	145.9 $\pm$ 2.8*
PdBu (300 nM)	298.2 $\pm$ 32.1**
PdBu (300 nM) + staurosporine (100 nM)	88.6 $\pm$ 13.3

were treated with increasing concentrations of BK for 2 h in serum-free medium. Proteins collected in the conditioned medium were analysed by Western blot with sAPP-specific antibodies. sAPP was detected as a protein with an apparent molecular mass of 105/110 kDa. As shown in Figure 1(A), release of sAPP from the fibroblasts was elicited by the treatment with BK in a concentration-dependent manner, reaching maximal stimulation averaging 2.4-fold of basal release at a BK concentration of 1 μM. Experiments performed with a wider range of concentrations allowed the construction of a concentration–response

Figure 2 Dose-dependent effect of BK on APP secretion from control (non-AD) and AD fibroblasts

Fibroblasts from six AD and five control (non-AD) subjects were incubated in serum-free medium for 2 h at 37 °C with increasing concentrations of BK ranging from 0.1 to 100 nM. Data are the result of densitometric analysis of Western-blot immunoreactive bands and are expressed as percentages over basal APP secretion: each point represents the mean $\pm$ S.E.M. of data from at least four different cell lines.

curve as shown in Figure 1(B). The mean EC₅₀ for BK-mediated sAPP release estimated from the curve was 2.8 nM.

The increase in sAPP secretion could be stimulated by treatment with the specific kinin receptor subtype 2 (B2 receptor) subtype agonist [Hyp³]BK (Table 1). The response to 10 nM [Hyp³]BK was quantitatively similar to that of 3 nM BK at a concentration consistent with the difference in agonist potency [32]. The effect of BK was inhibited by simultaneous treatment with the specific B2 subtype receptor antagonist HOE140. The compound alone did not have any intrinsic effect on sAPP release, but was able to inhibit sAPP release induced by BK to basal levels or lower. All these data support a receptor-mediated effect of BK on sAPP release (Table 1).

The effect of BK on APP metabolism was, however, insensitive to PKC inhibition. Treatment with the phorbol ester PdBu (150 nM) induced, as shown previously [28], a 2.9-fold increase (range 2.4–3.5) in sAPP release from human skin fibroblasts. The effect of PdBu was completely inhibited by treatment with staurosporine (100 nM). The kinase inhibitor, however, failed to abolish the response to 3 nM BK, suggesting that activation of PKC is not necessary for the secretory effect of BK (Table 1).

We next investigated whether the increase in IP₃ production after BK treatment described in fibroblasts from AD patients [27] would be mirrored by an increased sensitivity to BK in terms of sAPP secretion. Cells from six AD patients and five age-matched non-AD subjects (mean age 70.0 $\pm$ 8.2 and 68.2 $\pm$ 3.8 years respectively) were treated with increasing concentrations of BK ranging from 0.1 to 100 nM, and sAPP release was followed in the conditioned medium as described above. The concentration-dependent release of sAPP after BK treatment revealed no difference between AD and non-AD cells (Figure 2). The EC₅₀ values of BK-mediated sAPP release estimated from the curves shown in Figure 2 were 1.8 and 2.0 nM for AD and non-AD cells respectively, not statistically different from the K_d estimated for fibroblasts from young healthy volunteers.

We then analysed IP₃ production after BK treatment in the

Figure 3 IP₃ [Ins(1,4,5)P₃] levels in control and AD fibroblasts after BK treatment

Fibroblasts from control (non-AD) donors and AD patients were treated with 1 μ M BK for 10 s at 37 °C. Each point represents the mean of two independent determinations of triplicate samples for each cell line. The horizontal line represents the mean of each group; the means of the two groups were not significantly different.

same set of cell lines. Pilot experiments showed that treatment with 1 μ M BK elicited a rapid (peak within 5–10 s) increase in intracellular IP₃ levels followed by a slow decrease (not shown). For the comparison between AD and non-AD patients, cells were treated with 1 μ M BK for 10 s, and IP₃ was measured by a radioreceptor-binding assay. Levels of IP₃ did not differ in the two groups of cells in contrast with previously reported data. IP₃ production was slightly lower in AD fibroblasts, but on average there was no statistical significance (Figure 3). Basal levels of IP₃ were also not statistically different (results not shown).

DISCUSSION

The results described are the first evidence of receptor-mediated modulation of sAPP secretion in primary human skin fibroblasts. We show here that, in primary human skin fibroblasts, which are known to express endogenously the B₂ subtype of BK receptors [33], BK treatment promotes a concentration-dependent increase in sAPP release with an EC₅₀ of 2.8 nM, which is consistent with the previously reported K_d of BK binding [27,33]. Direct interaction of the peptide agonist with the B₂ subtype receptor is further supported by the activity of [Hyp³]BK, a specific B₂ agonist, which has a secretory effect similar to that of BK. Furthermore, the action of BK is antagonized by HOE140, a specific B2 receptor antagonist [34]. Treatment with the antagonist alone consistently failed to produce any effect on the basal release of sAPP, demonstrating the lack of any intrinsic activity of the compound. More puzzling was the finding that simultaneous treatment with BK and HOE140 in some cases inhibited sAPP release to below basal levels. The biological significance of this effect is not known and needs to be further characterized.

The observation that staurosporine did not affect BK-mediated sAPP release is rather interesting. Although BK treatment of human foreskin fibroblasts has been shown to promote translocation of PKC [33] activation of PKC through this pathway does not seem to be directly involved in sAPP secretion. Other authors have suggested the existence of PKC-independent mechanisms regulating APP metabolism, demonstrating that PKC-mediated signal-transduction pathways are not exclusively involved in the induction of sAPP release through stimulation of m3 receptors [35] or 5HT_{2a} and 5HT_{2c} receptors [15]. However, these data were obtained using cells transfected with the receptors

of interest, whereas our data are the first example of the co-existence of a PKC-dependent and a PKC-independent mechanism for sAPP secretion in primary human skin fibroblasts.

Although in most cell types the activation of sAPP secretion is often associated with a reduction in A β release, we were not able to measure the release of A β from fibroblasts in the same experimental paradigm as was used for the detection of sAPP. As shown previously ([36]; M. Racchi, unpublished work), the detection of A β released from cultured skin fibroblasts requires a completely different experimental design with incubation periods of longer than 24 h. The role of BK in the release of A β remains to be investigated following the design of an appropriate experimental approach.

Multiple signal-transduction systems are altered in cultured fibroblasts from AD patients, of which alterations in PKC activity is one of the most reproducible findings [23–25]. Other alterations in transduction systems involve the response to BK stimulation in terms of IP₃ release. Increased production of IP₃ after BK treatment in AD fibroblasts has been reported, which correlated with an increased number of BK receptors [27]. In our investigation, although we carefully replicated the methods described in the original paper, we failed to observe any difference in the response of AD and non-AD fibroblasts to BK by measuring either IP₃ production or sAPP release. A possible explanation for the observed discrepancies can be formulated by examining the sets of cell lines used in the two studies. Patients enrolled in our study were specifically selected through clinical anamnestic evaluation to fit into the group of ‘sporadic’ cases. Familial cases are often characterized by the presence of missense mutations either on chromosome 21 in the APP gene or on chromosome 14 on a gene coding for a 45–50 kDa protein named presenilin 1 (PS1) as well as in the homologue presenilin 2 gene on chromosome 1 (for a review see ref. [37]). Indeed seven of the ten cell lines employed in the former study [27] were derived from patients with a familial history of dementia, as indicated in other publications [36,38–40] using the same cell lines from the Coriell Cell Repository. More specifically, four cell lines were shown to be carrier of a mutation on PS1 (A246E), found in the Canadian FAD1 pedigree.

The contradictory findings described here can be explained by assuming that mutations present in the set of cells used in Huang’s study may have affected IP₃ responses and BK receptor expression. Since little is known about the normal function of presenilins, it is difficult to speculate on how mutations in these genes can affect cellular homeostasis.

As an example, it has been shown that mutations in different domains of PS1 can differentially affect the signal-transduction cascade elements in fibroblasts, resulting in either decreased isoprenaline-stimulated adenylate cyclase activity [26] or a statistically significant increase in adenylate cyclase activity [41] after β -adrenergic-receptor stimulation.

These observations and the data presented here suggest the possibility that the alteration in multiple signal-transduction systems in familial and sporadic AD fibroblasts (PKC activity, inositol turnover and adenylate cyclase activity) can assume divergent phenotypical patterns as a function of the presence or absence of mutations and also be distinguished as a function of specific PS1 mutations present.

Although these can be viewed as suggestive speculations, more work needs to be carried out to exclude clearly the influence of all methodological variables including the reproducibility of results obtained in different laboratories. The data described here were obtained on a set of cell lines characterized previously as having defective PKC-mediated sAPP release. Within the same set of cells, we now report the existence of a receptor-mediated

cellular mechanism leading to sAPP secretion that is independent of PKC and that is not defective in AD fibroblasts.

We are grateful to Dr. Werner Mueller-Esterl for the gift of HOE140. We also thank Dr. Gary Gibson for helpful discussion and careful reading of the manuscript. This work was partially funded by a CNR (Consiglio Nazionale delle Ricerche) grant to M.R.

REFERENCES

- Kang, J., Lemaire, H., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K. K., Multhaup, G., Beyreuther, K. and Müller-Hill, B. (1987) *Nature (London)* **325**, 733–736
- Weidemann, A., König, G., Bunke, D., Fischer, P., Salbaum, J. M., Masters, C. L. and Beyreuther, K. (1989) *Cell* **57**, 115–126
- Sisodia, S. S., Koo, E. H., Beyreuther, K., Unterbeck, A. and Price, D. L. (1990) *Science* **248**, 492–495
- Sisodia, S. S. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 6075–6079
- Esch, F. S., Keim, P. S., Beattie, E. C., Blacher, R. W., Culwell, A. R., Oltersdorf, T., McClure, D. and Ward, P. J. (1990) *Science* **248**, 1122–1124
- Haass, C., Schlossmacher, M. G., Hung, A. Y., Vigo-Pelfrey, C., Mellon, A., Ostaszewski, B. L., Lieberburg, I., Koo, E. H., Schenk, D., Teplow, D. B. and Selkoe, D. J. (1992) *Nature (London)* **359**, 322–324
- Shoji, M., Golde, T. E., Ghiso, J., Cheung, T. T., Estus, S., Shaffer, L. M., Cai, X., McKay, D. M., Tintner, R., Frangione, B. and Younkin, S. G. (1992) *Science* **258**, 126–129
- Checler, F. (1995) *J. Neurochem.* **65**, 1431–1444
- Buxbaum, J. D., Gandy, S. E., Cichetti, P., Ehrlich, M. E., Czernik, A. J., Fracasso, R. P., Ramabhadran, T. V., Unterbeck, A. J. and Greengard, P. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6003–6006
- Caporaso, G. L., Gandy, S. E., Buxbaum, J. D., Ramabhadran, T. V. and Greengard, P. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 3055–3059
- Gillespie, S. L., Golde, T. E. and Younkin, S. G. (1992) *Biochem. Biophys. Res. Commun.* **187**, 1285–1290
- Buxbaum, J. D., Oishi, M., Chen, H. I., Pinkas-Kramarski, R., Jaffa, E. A., Gandy, S. E. and Greengard, P. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10075–10078
- Nitsch, R. M., Slack, B. E., Wurtman, R. J. and Growdon, J. H. (1992) *Science* **258**, 304–307
- Wolf, B. A., Wertkin, A. M., Jolly, Y. C., Yasuda, R. P., Wolfe, B. B., Konrad, R. J., Manning, D., Ravi, S., Williamson, J. R. and Lee, V. M. (1995) *J. Biol. Chem.* **270**, 4916–4922
- Nitsch, R. M., Deng, M., Growdon, J. H. and Wurtman, R. J. (1996) *J. Biol. Chem.* **271**, 4188–4194
- Nitsch, R. M., Slack, B. E., Farber, S. A., Borghesani, P. R., Schulz, J. G., Kim, C., Felder, C. C., Growdon, J. H. and Wurtman, R. J. (1994) *Ann. N.Y. Acad. Sci.* **881**, 122–127
- Hung, A. H., Haass, C., Nitsch, R. M., Qiu, W. Q., Citron, M., Wurtman, R. J., Growdon, J. H. and Selkoe, D. J. (1993) *J. Biol. Chem.* **268**, 22959–22962
- Buxbaum, J. D., Koo, E. H. and Greengard, P. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 9195–9198
- Jacobsen, J. S., Spruyt, M. A., Brown, A. M., Sahasrabudhe, S. R., Blume, A. J., Vitek, M. P., Muenkel, H. A. and Sonnenberg-Reines, J. (1994) *J. Biol. Chem.* **269**, 8376–8382
- Saitoh, T., Horsburgh, K. and Masliah, E. (1993) *Ann. N.Y. Acad. Sci.* **69**, 34–41
- Gibson, G., Martins, R., Blass, J. and Gandy, S. (1996) *Life Sci.* **59**, 477–489
- Govoni, S., Gasparini, L., Racchi, M. and Trabucchi, M. (1996) *Life Sci.* **59**, 461–468
- Van Huynh, T., Cloe, G., Sundsmo, M., Katzman, R. and Saitoh, T. (1989) *Arch. Neurol.* **46**, 1195–1199
- Bruel, A., Cherqui, G., Columelli, S., Margelin, D., Roudier, M., Sinet, P. M., Prieur, M., Pérignon, J. L. and Delabar, J. (1991) *Neurosci. Lett.* **133**, 89–92
- Govoni, S., Bergamaschi, S., Racchi, M., Battaini, F., Binetti, G., Bianchetti, A. and Trabucchi, M. (1993) *Neurology* **43**, 2581–2586
- Huang, H.-M. and Gibson, G. E. (1993) *J. Biol. Chem.* **268**, 14616–14621
- Huang, H. M., Lin, T. A., Sun, G. Y. and Gibson, G. E. (1995) *J. Neurochem.* **64**, 761–766
- Bergamaschi, S., Binetti, G., Govoni, S., Wetsel, W. C., Battaini, F., Trabucchi, M., Bianchetti, A. and Racchi, M. (1995) *Neurosci. Lett.* **201**, 1–4
- McKhann, G., Drachman, D., Folstein, M. F., Katzman, R., Price, D. and Stadlan, E. M. (1984) *Neurology* **34**, 939–944
- Folstein, M. F., Folstein, S. E. and McHugh, P. R. (1975) *J. Psychiatr. Res.* **12**, 189–198
- Tesco, G., Vergelli, M., Amaducci, L. and Sorbi, S. (1993) *Exp. Gerontol.* **28**, 51–58
- Regoli, D., Gobeil, F., Nguyen, Q. T., Jukic, D., Seoane, P. R., Salvino, J. M. and Sawatz, D. G. (1994) *Life Sci.* **55**, 735–749
- Tippmer, S., Quitterer, U., Kolm, V., Faussner, A., Roscher, A., Mosthaf, L., Müller-Esterl, W. and Haring, H. (1994) *Eur. J. Biochem.* **225**, 297–304
- Hock, F. J., Whirt, K., Albus, U., Linz, W., Gerhards, H. J., Wiemer, G., Henke, S., Breipohl, G., König, W., Knolle, J. and Schölkens, B. A. (1991) *Br. J. Pharmacol.* **102**, 769–773
- Buxbaum, J. D., Ruef, A. A., Parker, C. A., Cypess, A. M. and Greengard, P. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 4489–4493
- Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T. D., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levy-Lahad, E., Viitanen, M., Peskind, E., Poorkaj, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D. and Younkin, S. (1996) *Nat. Med. N.Y.* **2**, 864–870
- Sandbrink, R., Hartmann, T., Masters, C. L. and Beyreuther, K. (1996) *Mol. Psychiatry* **1**, 27–40
- Kim, C. S., Han, Y. F., Etcheberrygaray, R., Nelson, T. J., Olds, J. L., Yoshioka, T. and Alkon, D. L. (1995) *Proc. Natl. Acad. Sci. U.S.A.* **92**, 3060–3064
- Ito, E., Oka, K., Etcheberrygaray, R., Nelson, T. J., McPhie, D. L., Tofel-Grehl, B., Gibson, G. E. and Alkon, D. L. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 534–538
- Etcheberrygaray, R., Ito, E., Oka, K., Tofel-Grehl, B., Gibson, G. E. and Alkon, D. L. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8209–8213
- Vestling, M., Adem, A., Racchi, M., Gibson, G. E., Lannfelt, L. and Cowburn, R. F. (1997) *Neuroreport* **8**, 2031–2035