

Presenilin-2 Mutations Modulate Amplitude and Kinetics of Inositol 1,4,5-Trisphosphate-mediated Calcium Signals*

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Malcolm A. Leissring^{‡§¶}, Ian Parker^{||}, and Frank M. LaFerla^{‡§¶*}

From the Laboratories of [‡]Molecular Neuropathogenesis and ^{||}Cellular and Molecular Neurobiology, Department of Neurobiology and Behavior, [§]Center for the Neurobiology of Learning and Memory, [¶]Institute for Brain Aging and Dementia, University of California, Irvine, California 92697-4545

Mutations in the two presenilin genes (*PS1*, *PS2*) account for the majority of early-onset familial Alzheimer's disease (FAD) cases. Converging evidence from a variety of experimental systems, including fibroblasts from FAD patients and transgenic animals, indicates that *PS1* mutations modulate intracellular calcium signaling pathways. Despite the potential relevance of these changes to the pathogenesis of FAD, a comparable effect for *PS2* has not yet been demonstrated experimentally. We examined the effects of wild-type *PS2*, and both of the identified FAD mutations in *PS2*, on intracellular calcium signaling in *Xenopus* oocytes. Inositol 1,4,5-trisphosphate (IP₃)-evoked calcium signals were significantly potentiated in cells expressing either of the *PS2* mutations relative to wild-type *PS2*-expressing cells and controls. Decay rates of calcium signals were also significantly accelerated in mutant *PS2*-expressing cells in a manner dependent upon IP₃ concentration. The finding that mutations in both *PS1* and *PS2* modulate intracellular calcium signaling suggests that these disturbances may represent a common pathogenic mechanism of presenilin-associated FAD.

Most cases of familial Alzheimer's disease (FAD)¹ are linked to mutations in two related genes, *presenilin-1* and *presenilin-2* (*PS1*, *PS2*) (1). These genes, which diverged from a single ancestor, share a similar genomic organization and encode proteins that are 67% identical. *PS1* and *PS2* are multipass, integral membrane proteins localized predominantly to the

endoplasmic reticulum (ER) (2, 3) and are widely expressed throughout the brain (4). Despite their similarities, there are genetic and physiological differences between these two molecules that may reflect important functional differences. For instance, while over 50 mutations have been identified in *PS1*, only two mutations have been found in *PS2* that are definitively linked to FAD: one in a group of eight Volga German kindreds (*PS2N141I*) and another in an Italian kindred (*PS2M239V*) (5, 6). In addition, *PS1* mutations cause an aggressive form of FAD with a particularly early age of onset, whereas *PS2* is associated with a form of FAD that more closely resembles sporadic AD in terms of age of onset and disease duration (7). Physiologically, although *PS1* and *PS2* share in common certain features such as modulating A β production, there are also key distinctions. Regarding their effect on apoptosis, for example, wild-type *PS2* (wt*PS2*), but not *PS1*, has been shown to be proapoptotic (8–10). In addition, *PS1* and *PS2* exhibit contrasting roles in affecting neuronal differentiation *in vitro* (11). Therefore, the functions mediated by the presenilins cannot be assumed to be identical.

PS1 mutations alter intracellular calcium signaling pathways in a variety of experimental systems (10, 12–19). These changes may be highly relevant to the pathogenesis of AD, since calcium dysregulation can contribute to several key features of AD, including increased A β production (20, 21), hyperphosphorylation of tau (22), enhanced vulnerability to cell death (10, 23), and even memory-related deficits (24). However, despite the likely significance of these changes to the pathogenesis of AD, no studies have yet reported the effects of *PS2* mutations on calcium signaling.

Circumstantial experimental evidence supports the potential involvement of *PS2* in intracellular calcium regulation. For instance, *PS2* interacts with a calcium-binding protein, calsenilin, that has been shown to regulate the levels of the caspase-3 cleavage product of *PS2* (25). Although the function of calsenilin remains to be determined, sequence homology with neuronal calcium sensor-1 suggests that it may play a role in regulating intracellular calcium levels. Moreover, *PS2*, but not *PS1*, interacts with another calcium-binding protein known as sorcin (26). Sorcin is known to modulate the activity of ryanodine receptors (27), a major type of intracellular calcium release channel, and has also been found to associate with L-type voltage-gated calcium channels (28). Collectively, these findings suggest a possible physiological role for *PS2* in regulating calcium signaling pathways, a function that may be modulated by FAD mutations.

Here we studied the effects of wild-type *PS2* (wt*PS2*) and both of the identified FAD-linked *PS2* mutations on calcium signaling in *Xenopus* oocytes. We show that, relative to controls, both FAD mutations in *PS2* significantly potentiated calcium signals evoked by the ubiquitous intracellular second messenger inositol 1,4,5-trisphosphate (IP₃). Notably, both *PS2* mutations also significantly accelerated decay rates of calcium signals evoked by high levels of IP₃. These findings lend strong support to the hypothesis that alterations in calcium signaling pathways may be a common pathogenic mechanism by which presenilin mutations contribute to FAD.

EXPERIMENTAL PROCEDURES

cRNA Synthesis and Injection—Full-length cDNAs encoding human wt*PS2*, *PS2N141I*, and *PS2M239V* were the generous gift of Dr. John

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** To whom correspondence should be addressed: Dept. of Neurobiology & Behavior, University of California, Irvine, 1109 Gillespie Neuroscience Facility, Irvine, CA 92697-4545. Tel.: 949-824-1232; Fax: 949-824-7356; E-mail: laferla@uci.edu.

¹ The abbreviations used are: FAD, familial Alzheimer's disease; AD, Alzheimer's disease; c-IP₃, caged IP₃; ER, endoplasmic reticulum; IP₃, inositol 1,4,5-trisphosphate; *PS1* and *PS2*, presenilin-1 and presenilin-2; OG-5N, Oregon green-5N.

Hardy. Synthesis of m⁷G(5')ppp(5')G-capped cRNA was performed by run-off transcription of template plasmids linearized with the restriction endonuclease *Xba*I using the Riboprobe Gemini System (Promega) according to manufacturer's recommendations. The quantity and quality of the resulting transcripts were determined by spectrophotometric analysis and direct visualization on a denaturing gel as described elsewhere (17). Stage V and VI oocytes of *Xenopus laevis* (*Xenopus* I, Ann Arbor, MI) were defolliculated by two 1-h treatments with 0.5 mg/ml collagenase and were injected the following day with 46 nl of the appropriate cRNA (500 ng/μl) or RNase-free H₂O (17).

Injection with Calcium Indicator and Caged IP₃.—Three days after cRNA injection and 1–4 h prior to calcium imaging experiments, oocytes were loaded with 46 nl of a mixture containing 0.25 mM caged IP₃ (c-IP₃; D-*myo*-inositol 1,4,5-trisphosphate, P⁴⁽⁵⁾-1-(2-nitrophenyl)ethyl ester; Molecular Probes, Eugene, OR) and 1 mM of the low affinity calcium indicator Oregon Green-5N (OG-5N; Molecular Probes), yielding final concentrations in the oocytes of ~12 and ~46 μM, respectively. To ensure equal loading of the oocytes, we used a piston-driven Nanoject microinjector apparatus (Drummond Scientific) fitted with freshly pulled glass electrodes with tip diameters of ~15–20 μm.

Photolysis of c-IP₃ and Calcium Imaging.—Photolysis of c-IP₃ to liberate free intracellular IP₃ was achieved using flashes of UV light (340–400 nm) derived from a mercury arc lamp (17). The concentration of IP₃ photoreleased by UV light is a function of both flash duration, controlled by a mechanical shutter, and flash intensity, controlled by a neutral density filter (29). For experiments using supramaximal IP₃ stimulation, the neutral density filter was reduced so that further increases in UV intensity failed to generate increased calcium signals in response to a fixed (200 ms) flash duration. Remaining experiments used a neutral density setting giving a flash intensity ~57-fold weaker. Estimates of IP₃ concentrations (see legend to Fig. 2) were calculated based on previous findings indicating that ~50 nM IP₃ is required to initiate a calcium wave (29).

Calcium-dependent fluorescence changes of OG-5N in response to photoreleased IP₃ were imaged using a custom-built line-scanning confocal microscope, and data were recorded and analyzed as described previously (30). Fluorescence intensities were averaged over a 50-μm scan line and are expressed as the change in intensity over the basal level of fluorescence prior to stimulation ($\Delta F/F_o$). Statistical comparisons were made using two-factor analysis of variance with replication. All results are presented as mean values \pm S.E.

Western Blotting.—Immediately following completion of the calcium imaging experiments, cells were frozen for later processing. Protein extracts were prepared as described previously (17) and concentrations determined by the Bradford method. Equal amounts of protein (10 μg) were separated by SDS-polyacrylamide gel electrophoresis on 5 or 10% acrylamide gels, transferred to nitrocellulose membranes, blocked overnight in 5% non-fat milk in Tris-buffered saline (pH 7.5) supplemented with 0.2% Tween 20, and processed as described previously (17). The α PS2_N antibody (the generous gift of Dr. Carl Cotman) was generated in rabbits by immunization with a synthetic peptide corresponding to amino acids 8–21 of the PS2 protein, affinity-purified, and used at a dilution of 1:1000. Other antibodies and dilutions used in this study include PS2_{NT} (1:5000; the generous gift of Dr. Gopal Thinakaran; Ref. 31); PS2 loop antibody 3711 (1:500; the generous gift of Dr. Christian Haass; Ref. 32); and α IP₃R-I, a rabbit polyclonal anti-type-I IP₃ receptor antibody (Affinity Bioreagents, Golden, CO). Quantitative densitometric analyses were performed on digitized images of immunoblots using the Stratagene EagleEye II gel documentation system according to manufacturer's recommendations (Stratagene, La Jolla, CA).

RESULTS

IP₃-mediated Calcium Release Is Potentiated by PS2 Mutations.—To determine whether PS2 mutations modulate IP₃-mediated calcium signaling, we conducted fluorescent calcium imaging in *Xenopus* oocytes expressing wild-type or mutant PS2 and H₂O-injected controls (Fig. 1). Intracellular IP₃ was liberated from a caged precursor by flashes of UV light. By varying the flash durations, different relative amounts of IP₃ could be photoreleased in a reproducible manner. The rapid calcium signals evoked by photorelease of caged IP₃ are almost exclusively due to liberation of calcium from the ER through IP₃ receptors and negligible amounts of calcium influx from outside the cell occur at this time scale (33).

Relative to controls, cells expressing either PS2N141I or

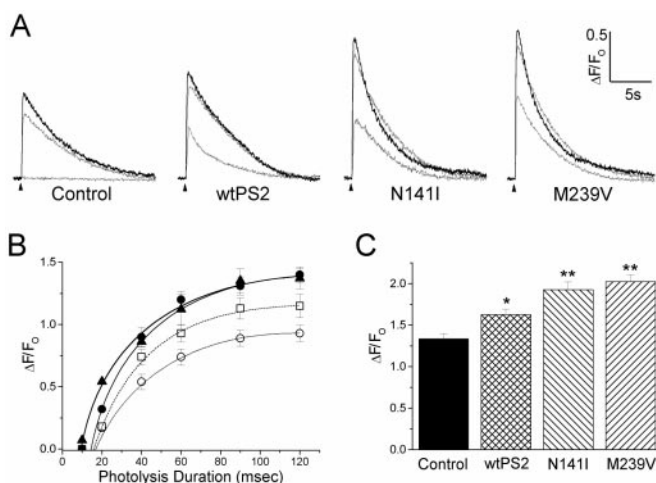


FIG. 1. PS2 mutations potentiate IP₃-evoked calcium liberation. A, sample recordings of calcium-dependent OG-5N fluorescence changes evoked by photoreleased IP₃ in single oocytes injected 3 days prior to recording with either H₂O or 25 ng of cRNA encoding either wtPS2, PS2N141I, or PS2M239V. Superimposed traces show responses evoked by 20-, 60-, and 120-ms flash durations, expressed as the change in fluorescence intensity relative to baseline ($\Delta F/F_o$). Arrowheads indicate approximate time of flash onset. Note that responses elicited by 120-ms flashes (**bold**) are markedly accelerated in mutant PS2-expressing cells, but not in wtPS2-expressing cells or controls, relative to signals evoked by shorter flash durations. B, mean peak $\Delta F/F_o$ plotted as a function of flash duration (i.e. [IP₃]) in control cells (○), or cells expressing wtPS2 (□), PS2N141I (●), or PS2M239V (▲) ($n = 8$ –10 oocytes per condition taken from a single donor frog). C, quantitative comparison of mean peak $\Delta F/F_o$ in response to maximal IP₃ stimulation in cells expressing each of the PS2 constructs or controls ($n = 28$ –30 cells per condition from two donor frogs). All values represent means \pm S.E., although S.E. values were omitted where data were not normally distributed; *, $p < 0.05$ relative to H₂O-injected controls; **, $p < 0.001$ relative to H₂O-injected controls and $p < 0.05$ relative to wtPS2-expressing cells.

PS2M239V exhibited larger mean peak responses for each flash duration tested, whereas wtPS2-expressing cells produced responses intermediate between mutant-expressing and control cells (Fig. 1, A and B). Moreover, with increasing flash duration, mean peak calcium responses approached higher asymptotic maxima in mutant PS2-expressing cells relative to controls (e.g. for 120-ms flashes, $\Delta F/F_o = 1.40 \pm 0.08$ versus 0.93 ± 0.06 for PS2N141I-injected cells versus controls, respectively; $p < 0.001$; Fig. 1B). To ensure that absolute maximal levels of IP₃-mediated calcium release had been achieved, cells were further stimulated with supramaximal [IP₃] (~30 μM; see “Experimental Procedures”). As with lower stimulus intensities, mean peak calcium responses following supramaximal IP₃ stimulation were significantly increased in cells expressing PS2 mutations relative both to wtPS2-expressing cells and to controls (Fig. 1C). Therefore, PS2 mutations consistently increase the mean amplitude of calcium responses evoked by a wide range of IP₃ levels.

The threshold for IP₃-mediated calcium release was lower in cells expressing PS2 mutations relative to controls, since we found that mutant-expressing cells responded more frequently than controls to the lowest flash durations tested. For example, in response to flash durations of 20 ms, six out of nine cells expressing PS2N141I responded as compared with only 1 of 10 H₂O-injected controls ($p < 0.001$ by χ^2 analysis). PS2M239V-expressing cells displayed even more marked differences, being the only cells to respond to flash durations of 10 ms (2 out of 10 trials; see Fig. 1B). Taken together, these data suggest that PS2 mutations confer an increased sensitivity to IP₃.

PS2 Mutations Accelerate Decay Rates of Calcium Signals.—We also examined the effects of PS2 mutations on the

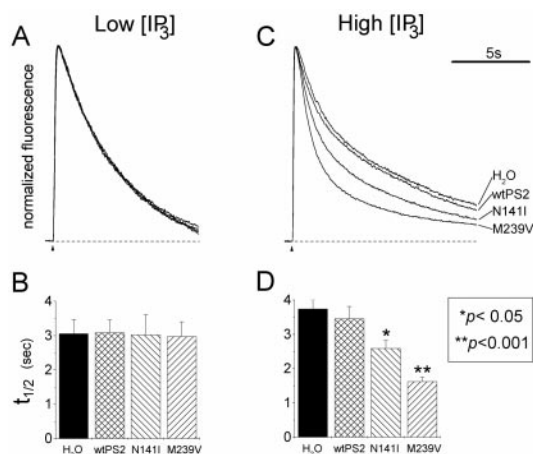


FIG. 2. PS2 mutations accelerate decay rates of calcium signals evoked by high, but not low, [IP₃]. A and C, average calcium responses evoked by “low [IP₃]” (A; $\sim 0.15 \mu\text{M}$) or “high [IP₃]” (C; $\sim 30 \mu\text{M}$) in cells expressing either *wtPS2*, *PS2N141I*, or *PS2M239V*. Traces represent the averages of 10 responses per condition, that have been normalized and superimposed for comparison. B and D, quantitation of mean times to decay from peak $\Delta F/F_0$ to one-half peak ($t_{1/2}$) for all recordings ($n = 28\text{--}30$ per condition) following stimulation with low or high [IP₃], respectively. Note that decay rates of calcium signals in mutant *PS2*-expressing cells are accelerated at high, but not low, [IP₃]; *wtPS2*-expressing cells did not differ significantly from controls. *, $p < 0.05$ relative to H₂O-injected controls; **, $p < 0.001$ relative to H₂O-injected controls and $p < 0.05$ relative to *PS2N141I*-expressing cells.

decay rates of calcium signals evoked by different concentrations of IP₃, which were quantitated by measuring the half-decay time ($t_{1/2}$) of individual signals (Fig. 2). At low [IP₃] ($\sim 0.15 \mu\text{M}$; see “Experimental Procedures”), calcium responses in wild-type and mutant *PS2*-expressing cells and controls decayed at similar rates (Fig. 2, A and B). By comparison, following supramaximal IP₃ stimulation (“high [IP₃]”; $\sim 30 \mu\text{M}$), decay rates were significantly slowed in *wtPS2*-expressing cells and controls (which were not significantly different from each other; Fig. 2, C and D). In striking contrast, decay rates were significantly accelerated in mutant *PS2*-expressing cells following supramaximal IP₃ stimulation relative to *wtPS2*-expressing cells and controls (Fig. 2, C and D; see also *bold traces* in Fig. 1A).

Expression of PS2 and IP₃ Receptor Proteins—To ascertain if differences in calcium signaling were due to differences in PS2 expression levels, protein extracts from the oocytes used for calcium imaging experiments were subjected to Western analysis using several anti-PS2 antibodies (Fig. 3A). For each antibody tested, protein expression levels did not differ by more than 5% among the *PS2* cRNA-injected conditions, as determined by densitometric analysis. Therefore, the increased potentiation observed in mutant *PS2*-expressing cells relative to wild-type *PS2*-expressing cells is not attributable to differences in protein expression levels. Interestingly, some antibodies, including the αPS2_N antibody (Fig. 3A), also detected a weak band in the H₂O-injected controls, presumably reflecting endogenous *Xenopus* presenilin (34).

To address the mechanism by which *PS2* mutations potentiate IP₃-mediated calcium release, we also conducted a Western analysis of type-I IP₃ receptors (Fig. 3B), which are the only type of calcium release channel expressed in *Xenopus* oocytes (35). IP₃ receptor protein levels differed only slightly ($\sim 7\%$) between control cells and cells expressing wild-type or mutant *PS2*, and these small differences did not correlate consistently with the amplitudes of calcium responses in the various conditions. These findings indicate that mechanisms other than IP₃ receptor up-regulation are responsible for the observed potentiation in IP₃-mediated calcium signaling.

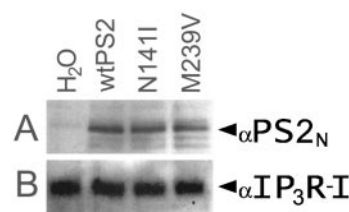


FIG. 3. Potentiation of calcium signaling is not attributable to differences in PS2 or IP₃ receptor protein expression levels. Immunoblots of protein extracts from control cells and cells injected with *wtPS2* or *PS2N141I* or *PS2M239V* cRNA. A, representative immunoblot of PS2 protein levels using antibody αPS2_N : levels of PS2 protein did not differ by more than 5% as determined by densitometric analysis. Similar results were obtained using several other well characterized anti-PS2 antibodies (see “Experimental Procedures”). Note the presence of a faint band in the H₂O-injected cells which may represent *Xenopus* presenilin. B, levels of type-I IP₃ receptors detected with antibody $\alpha\text{IP}_3\text{R-I}$ (Affinity Bioreagents). Protein levels differed only slightly ($< 7\%$), and these differences do not correlate with differences in mean amplitudes of calcium signals exhibited by cells in the various conditions.

DISCUSSION

The “calcium hypothesis” of AD proposes that perturbed intracellular calcium homeostasis plays a central role in AD neurodegeneration (36). In support of the idea, several distinct AD-linked *PS1* mutations have been shown to modulate calcium signaling in a variety of systems, including fibroblasts from FAD patients (12, 15, 16), mutant *PS1* “knock-in” mice (18), transgenic mice (19), transfected PC-12 cells (10, 14), and *Xenopus* oocytes (17). However, to our knowledge, the effects of *PS2* mutations on calcium signaling have not previously been examined. We addressed this question by measuring IP₃-mediated calcium signals in *Xenopus* oocytes expressing wild-type *PS2* and the two identified FAD-linked mutations in *PS2*. Calcium signals were potentiated in oocytes overexpressing wild-type *PS2* as compared with H₂O-injected controls, and a further potentiation was observed in oocytes expressing similar amounts of mutant *PS2*. Notably, the nature and extent of this potentiation closely resembles the results we observed for mutant *PS1* using identical methods in the same system (17). Therefore, FAD mutations in both *PS1* and *PS2* lead to similar alterations in IP₃-mediated calcium signaling, providing further evidence that intracellular calcium dysregulation may represent a common pathophysiological consequence of all AD-linked presenilin mutations. Since elevated cytosolic calcium levels can lead to several hallmark features of AD, including increased levels of A β (20, 21), hyperphosphorylated tau (22), and enhanced susceptibility to cell death (10, 23), these findings lend additional support to the hypothesis that calcium dysregulation plays a causative role in the pathogenesis of AD. Conversely, our data do not reveal differences in IP₃-mediated calcium signaling that may account for the contrasting functional differences between the *PS1* and *PS2* proteins.

Oocytes expressing wild-type *PS2* exhibited potentiated IP₃-mediated calcium release relative to H₂O-injected controls, albeit to a lesser extent than those expressing the FAD mutations. Overexpression of wild-type *PS1* in the same system also produced a comparable potentiation in IP₃-mediated calcium release (17). Although the significance of these findings is not presently clear, these results may reflect a physiological function of endogenous presenilins in calcium signaling, a function that is perturbed by FAD mutations. Given the interaction of the presenilins with calsenilin (25), which is homologous to proteins involved in calcium homeostasis, and sorcin, which modulates calcium release through ryanodine receptors, this possibility warrants further investigation.

Although the mechanism by which *PS2* mutations potentiate

IP₃-mediated calcium signaling remains to be established, up-regulation of IP₃ receptors does not appear to be involved, since comparable levels of IP₃ receptor levels were present in control cells and in cells expressing wild-type and mutant PS2. Alternative possibilities may involve alteration of IP₃-receptor function or overfilling of the ER calcium pools. In support of the latter idea, measurements of store content obtained by using thapsigargin to release calcium show an enhancement in PC-12 cells expressing mutant PS1 (14).

Rates of decay of calcium signals evoked by supramaximal [IP₃] were also markedly accelerated by PS2 mutations. The decay of the fluorescent calcium signal represents a balance between calcium efflux through activated IP₃ receptors and mechanisms that remove calcium ions from the cytosol (e.g. reuptake into the ER, uptake into mitochondria, extrusion across plasma membrane). Although we cannot entirely exclude effects of PS2 mutations on calcium sequestration mechanisms, it seems unlikely that this could result in an acceleration of calcium transport at high, but not low, [IP₃]. One mechanism that may account for these results might be an increase in the rate of inactivation of IP₃ receptors in the presence of high [IP₃]. Alternatively, the accelerated decay rates might be due to the increased calcium release evoked by high [IP₃] in the mutant PS2-expressing cells, since it is known that high local levels of cytosolic calcium can actually inhibit the opening of IP₃ receptors (37).

The fact that intracellular calcium signaling is modulated by mutations in both PS1 and PS2 increases support for the hypothesis that these disturbances play a causative role in the pathogenesis of FAD. In view of the central importance of intracellular calcium regulation for numerous cellular processes, elucidation of the mechanisms responsible for these changes is clearly warranted and could uncover novel targets for therapeutic intervention.

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REFERENCES

- Cruts, M., van Duijn, C. M., Backhovens, H., Van den Broeck, M., Wehnert, A., Serneels, S., Sherrington, R., Hutton, M., Hardy, J., St George-Hyslop, P. H., Hofman, A., and Van Broeckhoven, C. (1998) *Hum. Mol. Genet.* **7**, 43–51
- Kovacs, D. M., Fausett, H. J., Page, K. J., Kim, T. W., Moir, R. D., Merriam, D. E., Hollister, R. D., Hallmark, O. G., Mancini, R., Felsenstein, K. M., Hyman, B. T., Tanzi, R. E., and Wasco, W. (1996) *Nat. Med.* **2**, 224–229
- Zhang, J., Kang, D. E., Xia, W., Okochi, M., Mori, H., Selkoe, D. J., and Koo, E. H. (1998) *J. Biol. Chem.* **273**, 12436–12442
- Cribbs, D. H., Chen, L. S., Bende, S. M., and LaFerla, F. M. (1996) *Am. J. Pathol.* **148**, 1797–1806
- Levy-Lahad, E., Wasco, W., Poorkaj, P., Romano, D. M., Oshima, J., Pettingell, W. H., Yu, C. E., Jondro, P. D., Schmidt, S. D., Wang, K., and *et al.* (1995) *Science* **269**, 973–977
- Rogaev, E. I., Sherrington, R., Rogaeva, E. A., Levesque, G., Ikeda, M., Liang, Y., Chi, H., Lin, C., Holman, K., Tsuda, T., and *et al.* (1995) *Nature* **376**, 775–778
- Renbaum, P., and Levy-Lahad, E. (1998) *Cell. Mol. Life Sci.* **54**, 910–919
- Vito, P., Wolozin, B., Ganjei, J. K., Iwasaki, K., Lacana, E., and D'Adamio, L. (1996) *J. Biol. Chem.* **271**, 31025–31028
- Deng, G., Pike, C. J., and Cotman, C. W. (1996) *FEBS Lett* **397**, 50–54
- Guo, Q., Sopher, B. L., Furukawa, K., Pham, D. G., Robinson, N., Martin, G. M., and Mattson, M. P. (1997) *J. Neurosci.* **17**, 4212–4222
- Hong, C. S., Caromile, L., Nomata, Y., Mori, H., Bredesen, D. E., and Koo, E. H. (1999) *J. Neurosci.* **19**, 637–643
- Ito, E., Oka, K., Etcheberrigaray, 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
- Gibson, G. E., Zhang, H., Toral-Barza, L., Szolosi, S., and Tofel-Grehl, B. (1996) *Biochim. Biophys. Acta* **1316**, 71–77
- Guo, Q., Furukawa, K., Sopher, B. L., Pham, D. G., Xie, J., Robinson, N., Martin, G. M., and Mattson, M. P. (1996) *Neuroreport* **8**, 379–383
- Gibson, G. E., Vestling, M., Zhang, H., Szolosi, S., Alkon, D., Lannfelt, L., Gandy, S., and Cowburn, R. F. (1997) *Neurobiol. Aging* **18**, 573–580
- Etcheberrigaray, R., Hirashima, N., Nee, L., Prince, J., Govoni, S., Racchi, M., Tanzi, R. E., and Alkon, D. L. (1998) *Neurobiol. Dis.* **5**, 37–45
- Leisring, M. A., Paul, B. A., Parker, I., Cotman, C. W., and LaFerla, F. M. (1999) *J. Neurochem.* **72**, 1061–1068
- Guo, Q., Fu, W., Sopher, B. L., Miller, M. W., Ware, C. B., Martin, G. M., and Mattson, M. P. (1999) *Nat. Med.* **5**, 101–106
- Begley, J. G., Duan, W., Chan, S., Duff, K., and Mattson, M. P. (1999) *J. Neurochem.* **72**, 1030–1039
- Querfurth, H. W., and Selkoe, D. J. (1994) *Biochemistry* **33**, 4550–4561
- Querfurth, H. W., Jiang, J., Geiger, J. D., and Selkoe, D. J. (1997) *J. Neurochem.* **69**, 1580–1591
- Mattson, M. P., Engle, M. G., and Rychlik, B. (1991) *Mol. Chem. Neuropathol.* **15**, 117–142
- Mattson, M. P., Guo, Q., Furukawa, K., and Pedersen, W. A. (1998) *J. Neurochem.* **70**, 1–14
- Disterhoft, J. F., Moyer, J. R., Jr., and Thompson, L. T. (1994) *Ann. N. Y. Acad. Sci.* **747**, 382–406
- Buxbaum, J. D., Choi, E. K., Luo, Y., Lilliehook, C., Crowley, A. C., Merriam, D. E., and Wasco, W. (1998) *Nat. Med.* **4**, 1177–1181
- Kim, T.-W., Pettingell, W. P., and Tanzi, R. E. (1998) *Soc. Neurosci. Abstr.* **24**, 757
- Meyers, M. B., Pickel, V. M., Sheu, S. S., Sharma, V. K., Scotto, K. W., and Fishman, G. I. (1995) *J. Biol. Chem.* **270**, 26411–26418
- Meyers, M. B., Puri, T. S., Chien, A. J., Gao, T., Hsu, P. H., Hosey, M. M., and Fishman, G. I. (1998) *J. Biol. Chem.* **273**, 18930–18935
- Callamaras, N., and Parker, I. (1998) *Methods Enzymol.* **291**, 380–403
- Sun, X. P., Callamaras, N., Marchant, J. S., and Parker, I. (1998) *J. Physiol. (Lond.)* **509**, 67–80
- Saura, C. A., Tomita, T., Davenport, F., Harris, C. L., Iwatsubo, T., and Thinakaran, G. (1999) *J. Biol. Chem.* **274**, 13818–13823
- Walter, J., Grunberg, J., Schindzielorz, A., and Haass, C. (1998) *Biochemistry* **37**, 5961–5967
- Yao, Y., and Parker, I. (1993) *J. Physiol. (Lond.)* **468**, 275–295
- Tsujimura, A., Yasojima, K., and Hashimoto-Gotoh, T. (1997) *Biochem. Biophys. Res. Commun.* **231**, 392–396
- Kume, S., Muto, A., Aruga, J., Nakagawa, T., Michikawa, T., Furuichi, T., Nakade, S., Okano, H., and Mikoshiba, K. (1993) *Cell* **73**, 555–570
- Khachaturian, Z. S. (1987) *Neurobiol. Aging* **8**, 345–346
- Bezprozvanny, I., Watras, J., and Ehrlich, B. E. (1991) *Nature* **351**, 751–754