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Amyloid Precursor Protein Fragment and Acetylcholinesterase Increase with Cell Confluence and Differentiation in a Neuronal Cell Line

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This study addresses the developmental regulation of amyloid precursor protein (APP) fragments comprising the amyloid-β peptide (Aβ) and the amyloid-promoting factor acetylcholinesterase (AChE) in a mouse neuronal cell line (Neuro-2a). Results indicate that a 35-kDa amyloidogenic fragment of APP and the major molecular forms of AChE (G_1 and G_4) in Neuro-2a cells significantly increase with increasing levels of cell confluence. The foregoing molecules undergo further increases when neuroblastoma cells differentiate in the presence of dibutyryl cAMP. In contrast, a 17-kDa fragment of APP and butyrylcholinesterase were not affected by cell confluence or differentiation. These findings are the first to indicate that a selective Aβ-containing fragment of APP is subject to developmental regulation. Moreover, our data show that the 35-kDa fragment and AChE forms respond in parallel to the same developmental stimuli, i.e., cell confluence and differentiation. This points to the existence of a functional relationship between both molecules, a notion that is consistent with the potential role that has been ascribed to AChE in both APP processing and the formation of amyloid deposits in Alzheimer's brains.

INTRODUCTION

The significance of the elucidation of how nerve cells regulate the processing of amyloid precursor protein (APP) cannot be overemphasized; in fact, this highly conserved, widely expressed, integral membrane protein comprises the 4.1- to 4.3-kDa amyloid β-peptide (Aβ) that conforms the main component of amyloid deposits in senile plaques of patients suffering from Alzheimer's disease (AD) [1, 2]. Many efforts have been made to identify the functional significance of APP in various biological processes, including cell proliferation [3], axonal sprouting [4], and interaction with the extracellular matrix (ECM) [5]. Current knowledge suggests that the metabolism of APP involves two major pathways: an extracellular nonamyloidogenic route whereby transmembrane APP is cleaved between residues 16 and 17, precluding the formation of Aβ, and an intracellular amyloidogenic route yielding COOH-terminal fragments which contain the Aβ sequence [1]. The existence of several of these fragments [6] and their demonstrated precursor–product relationship with Aβ [7], suggest that the COOH-terminal region of APP participates in the targeting of this protein through the Aβ-producing amyloidogenic pathway.

Of particular relevance to the foregoing APP processing phenomena is the enzyme acetylcholinesterase (AChE) which catalyzes the hydrolysis of the neurotransmitter, acetylcholine, in the central and autonomic nervous systems [8, 9] and may also serve a function(s) in noncholinergic mechanisms [10]. The multiple oligomeric forms of AChE are not only profoundly affected by AD [11], but some of them also accumulate within the characteristic amyloid plaques [12]. In this regard, we have recently reported that the purified bovine brain tetrameric (G_4) AChE, as well as both human and mouse recombinant enzymes, effectively bind to soluble Aβ and promote the *in vitro* aggregation of this peptide into amyloid fibrils [13, 14]. Inasmuch as AChE-containing neurons are the most vulnerable cells in AD [15, 16], the above observations are consistent with the notion that AChE may be involved in the processing of membrane-bound APP and, thereby, play a role(s) in the formation of amyloid deposits *in vivo*.

Both APP and AChE are known to be expressed and regulated by nerve cells in culture [17–19]. Indeed, it has been shown that neuronal differentiation is accompanied by a decrease in the amount of secreted APP and a concomitant increase in the full-length membrane-associated protein [20, 21]; more recent work indicated that the total amount of APP was relatively constant over time [22]. It is documented that neurite outgrowth elicits an elevation of total AChE activity [23, 24], specific AChE molecular forms [18], and AChE mRNA [19].

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Further, it has been reported that in neuroblastoma (Neuro-2a) cells the formation of APP fragments containing the Aβ sequence is regulated by extracellular matrix components [25]. In the present work we have examined whether such APP fragments and the molecular forms of AChE expressed by Neuro-2a cells are influenced by cell confluence and differentiation.

MATERIALS AND METHODS

Cell cultures. Mouse C.1300 neuroblastoma cells (Neuro-2a clone) were maintained in Dulbecco's modified medium (DMEM) containing 5% (v/v) fetal calf serum, 100 U/ml penicillin, 2.5 mg/ml amphotericin B, 100 mg/ml streptomycin, and 3.7 g/liter $NaHCO_3$, pH 7.4 [24]. For experiments involving different stages of cellular confluence, the cells were seeded at initial densities of 9, 18, and 36 $\times 10^3$ cells/cm^2 and grown for 4 days. For experiments involving cellular differentiation, the cells were seeded at an initial density of 18×10^3 cells/cm^2; after 1 day the cultures were supplemented with 5 m*M* dibutyryl cyclic AMP (dbcAMP) and grown for an additional 3 days. In all cases, cells were harvested after 4 days in culture.

Preparation of samples. Cells were removed from the cultures flasks with 0.15 *M* phosphate-buffered saline (PBS), pH 7.4, containing 10 m*M* EDTA and centrifuged at 3000 rpm for 2 min in a clinical centrifuge (International Equipment Co). The pellets were resuspended and homogenized at 0°C in 300 μl of a solution containing 10 m*M* Tris–HCL, 0.1% Triton X-100, and a mixture of protease inhibitors. The homogenates were then centrifuged at 15,000 rpm for 15 min in a microcentrifuge (Eppendorf). Total protein content of supernatants were determined by the method of Lowry [26]. Supernatant samples were subsequently assayed for total AChE as well as butyrylcholinesterase (BuChE) activities, AChE molecular forms, and Aβ-bearing APP fragments.

Enzymatic assays. AChe activity was measured at 37°C for 30 min in a reaction mixture (1 ml total volume) containing 0.1 *M* Tris–HCL buffer (pH 7.2), 0.75 m*M* acetylthiocholine iodide as substrate, 0.3 m*M* 5,5′-dithio(bis)nitrobenzoic acid, and 0.1 m*M* tetraisopropyl pyrophosphoramide (Iso-OMPA) as an inhibitor of nonspecific cholinesterases. BuChE activity was measured under the above conditions, but with 5 m*M* butyrylthiocholine iodide as substrate and 10 μM BW284c51 dibromide as an inhibitor of AChE. The reactions were uniformly stopped by lowering the temperature of assay mixtures to 0°C [27].

Velocity sedimentation analyses. AChE molecular forms were evaluated by sedimentation on 5–20% linear sucrose gradients as described [18, 28]. In short, supernatant samples (200 μl) containing 400 μg of protein were individually layered on top of sucrose gradients containing 50 m*M* Tris–HCl (pH 7.4), 0.5% Triton X-100, 1 *M* NaCl, 3 m*M* EDTA, and protease inhibitors [29]. Approximately 45 fractions (100 μl) were collected from the bottom of each tube and individually assayed for AChE activity as described above. Sedimentation coefficients were estimated by comparison with that of catalase [11.3 S].

Analyses of APP fragments. Supernatant samples, each containing 60 μg of protein, were subjected to polyacrylamide gel electrophoresis (PAGE) in Tris–tricine buffer containing 16% polyacrylamide, 0.1% sodium dodecyl sulfate (SDS) [5, 30]. The proteins resolved by SDS–PAGE were either stained with Coomassie blue or electrophoretically transferred to nitrocellulose membranes [31]. Immunoblotting of the transferred proteins was performed in screening microtiter chambers by initially blocking any possible nonspecific binding of the primary antibody via incubation with nonfat dry milk in 10 m*M* Tris–HCl buffer (pH 8.0) containing 150 m*M* NaCl. Next, incubation was carried out overnight at 4°C with the SGY 2134 antibody directed against $A\beta_{1-40}$ (1:500). The APP antibody was used at a dilution 1:50. The samples were then incubated with an alkaline phosphatase-conjugated anti-rabbit IgG and immunoreactive bands identified by using 4 chloro-1-naphthol and 5-bromo-4-chloro 3-indolyl phosphate. Quantitation of individual bands was performed by densitometric scanning at 550 nm [25].

Antibodies. Antibodies used included a polyclonal antiserum, SGY2134, which binds the $A\beta_{1-40}$ peptide [32], a kind gift of Dr. Steven Younkin (Mayo Clinic, Jacksonville, FL). The APP polyclonal antiserum, anti-GID, which recognizes amino acids 175–186 of the N-terminal domain of the APP molecule [33], was kind gift of Dr. Gregory Cole (University of California, Los Angeles, CA).

Materials. Acetylthiocholine iodide, butyrylthiocholine iodide, IsoOMPA, BW284c51 dibromide, dbcAMP, DMEM, and antibiotics were all obtained from Sigma Chemical Co. (St. Louis, MO). Salts of the purest grade available were obtained from Merck.

RESULTS

Cellular Confluence and Differentiation

Undifferentiated Neuro-2a cells display distinct general morphologies when grown under conditions leading to different degrees of cellular confluence. As illustrated in Fig. 1, increasing initial seeding densities result in corresponding greater cellular confluence levels. At low levels these cells show flattened and expanded shapes, whereas at high levels they gradually become more rounded and exhibit enhanced cellular contact. In turn, when Neuro-2a cells are made to differentiate by supplementing the cultures with 5 m*M* dbcAMP they acquire their typical neural phenotype, with thin and elongated processes extending out from the cell bodies [24] (Fig. 2).

AChE and BuChE Activities

AChE in Neuro-2a cells gradually augments with greater levels of cellular confluence. As shown in Fig. 3A, total AChE activity is increased approximately sixfold at the highest compared to the lowest confluence levels studied. In contrast, under identical experimental condition, the amounts of total BuChE remained constant. Such a dramatic increase in AChE activity can be entirely accounted for by corresponding elevation in the two major molecular forms of the enzyme, i.e., the monomeric G_1 and the tetrameric G_4 species [18, 34] (Fig. 3B).

Similar results were obtained when Neuro-2a cells were made to differentiate in the presence of dbcAMP. As shown in Fig. 4A, total AChE activity increased approximately 2.8-fold in the differentiated versus control cells, whereas BuChE activity remained unchanged. Here again, the resulting increase in total AChE can be accounted for by corresponding enhancements in the enzyme's G_1 and G_4 forms (Fig. 4B). In this experimental series Neuro-2a cells were seeded at an initial density of 18×10^3 cells/cm^2; thus, the extent of elevation in total AChE activity induced by the differentiation process is comparable to that produced by

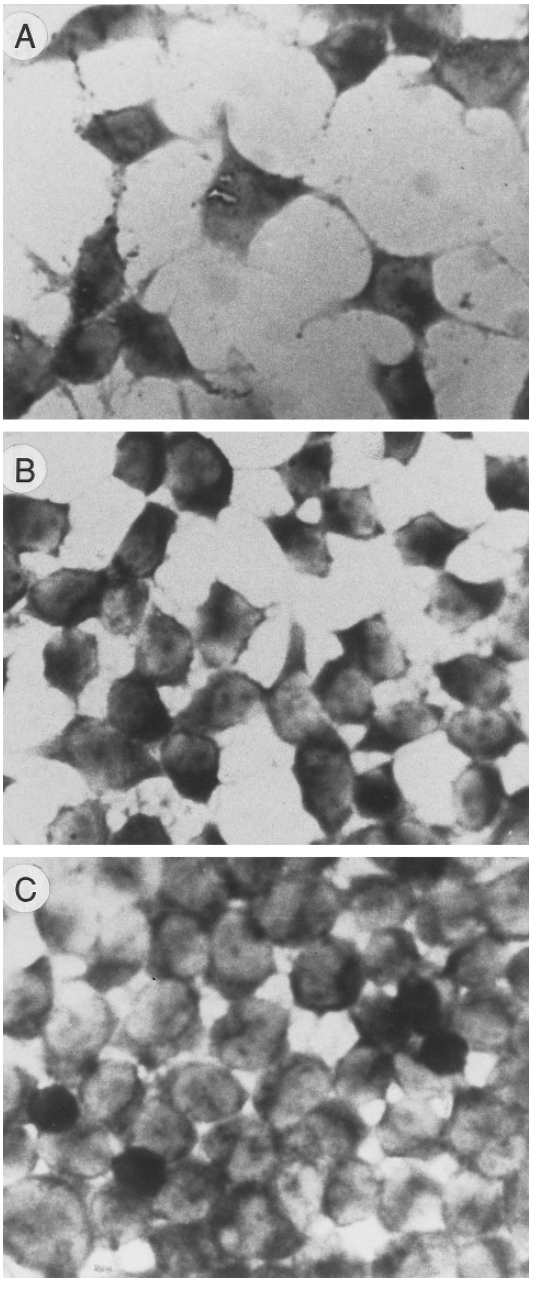

FIG. 1. General morphological features of undifferentiated Neuro-2a cells exhibiting different degrees of cellular confluence. After 4 days in culture (see Materials and Methods) the cells were fixed with 3% glutaraldehyde and stained with hematoxilin–eosin. Cells were initially seeded at: (A) 9×10^3 cells/cm^2, (B) 18×10^3 cells/cm^2, (C) 36×10^3 cells/cm^2. Magnification, ×4000.

cellular confluence of undifferentiated cells grown at the same initial seeding density (see Fig. 3A).

Aβ Peptide-Bearing APP Fragments

We have previously reported that Neuro-2a cells contain two amyloidogenic APP fragments of 17 and 35 kDa [25]. As shown in Fig. 5A, the 35-kDa fragment gradually increases with greater levels of cellular confluence, while the 17-kDa fragment remains constant. Quantitative densitometric analysis revealed that the

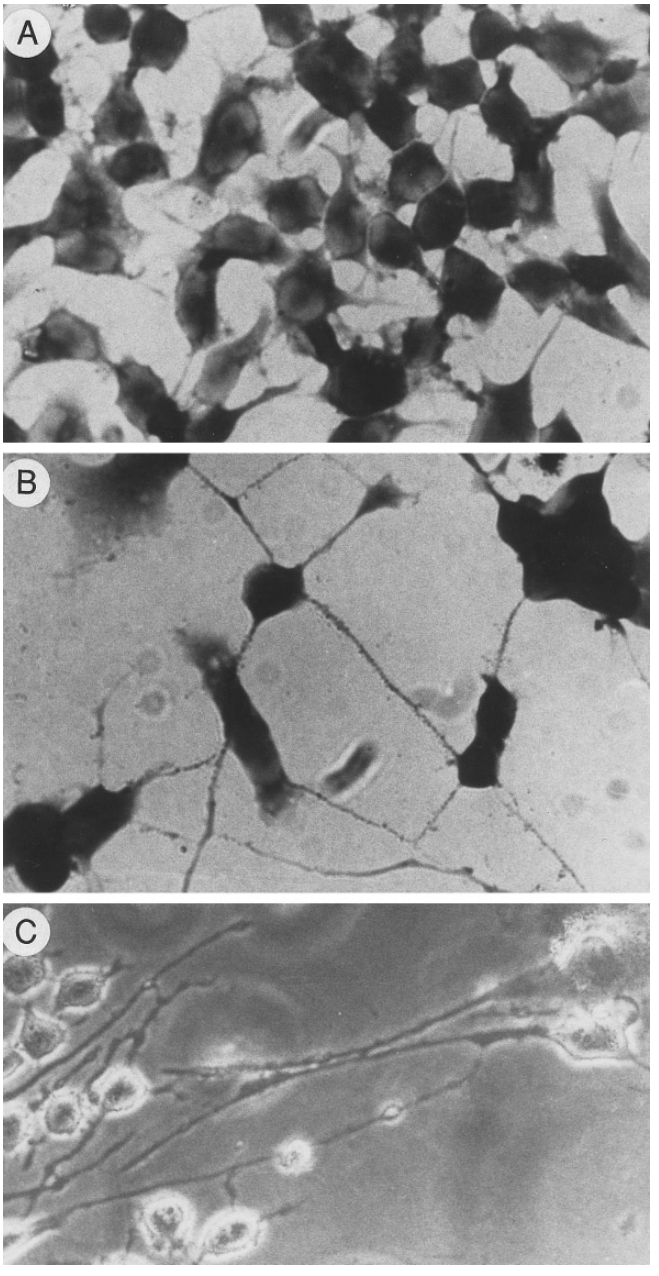

FIG. 2. Morphological features of differentiated Neuro-2a cells. Cells were initially seeded at a density of 18×10^3 cells/cm^2 and grown for 1 day. Subsequently, the cultures were supplemented with 5 m*M* dbcAMP to stimulate their differentiation and grown for an additional 3 days. After 4 days in culture (see Materials and Methods) the cells were fixed with 3% glutaraldehyde ans stained with hematoxilin–eosin. (A) Control cells grown in the absence of dbcAMP; (B) cells grown in the presence of dbcAMP; and (C) the same as (B) except that the cells were observed by phase-contrast microscopy. Magnification, ×4000.

35-kDa fragment increased approximately fivefold at the highest versus the lowest cellular confluence levels (Fig. 5B). In these experiments, the different degrees of cellular confluence did not alter the cell's general

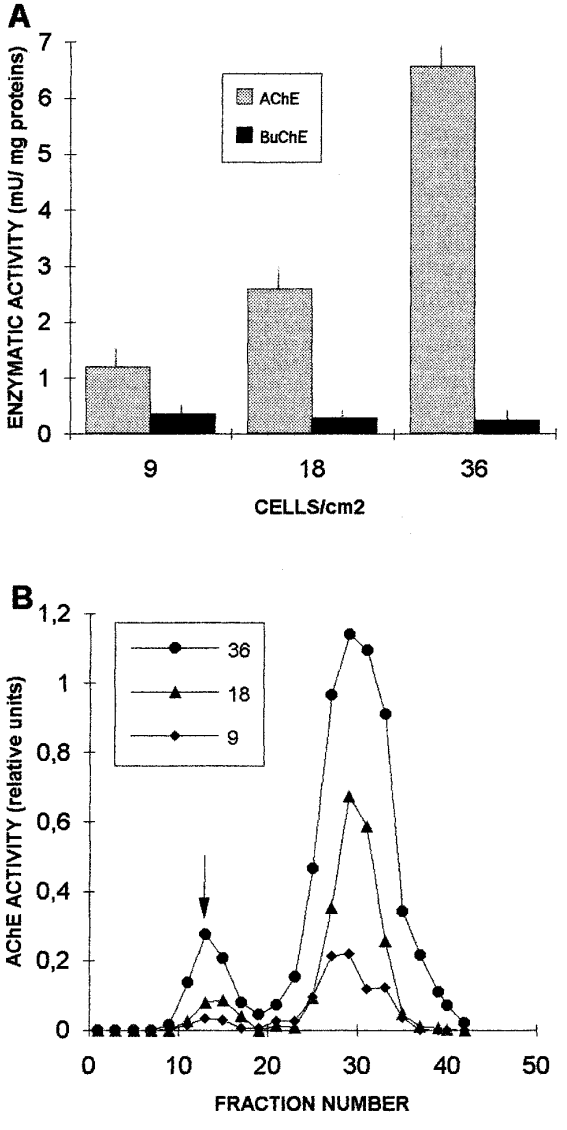

FIG. 3. AChE and BuChE in undifferentiated Neuro-2a cells exhibiting different degrees of cellular confluence. Cells were seeded at initial densities of 9×10^3 cells/cm^2, 18×10^3 cells/cm^2, and 36×10^3 cells/cm^2 and grown for 4 days (see Materials and Methods). (A) AChE and BuChE activities (mU/mg protein) as a function of the above initial seeding densities. One milliunit of AChE activity represents 1 n*M* of acetylthiocholine hydrolyzed/min. One milliunit of BuChE activity represents 1 n*M* of butyrylthiocholine hydrolyzed/min. Data are the mean ± SEM of three separate experiments in which the enzymatic activities were assayed in triplicate. (B) Velocity sedimentation profiles of AChE (relative units) extracted from cells at different stages of confluence. The arrow indicates the position of the sedimentation marker catalase (11.3S) and the meniscus is on the righthand side of the figure. Mayor peaks represent from right to left the G_1 and G_4 molecular forms of AChE.

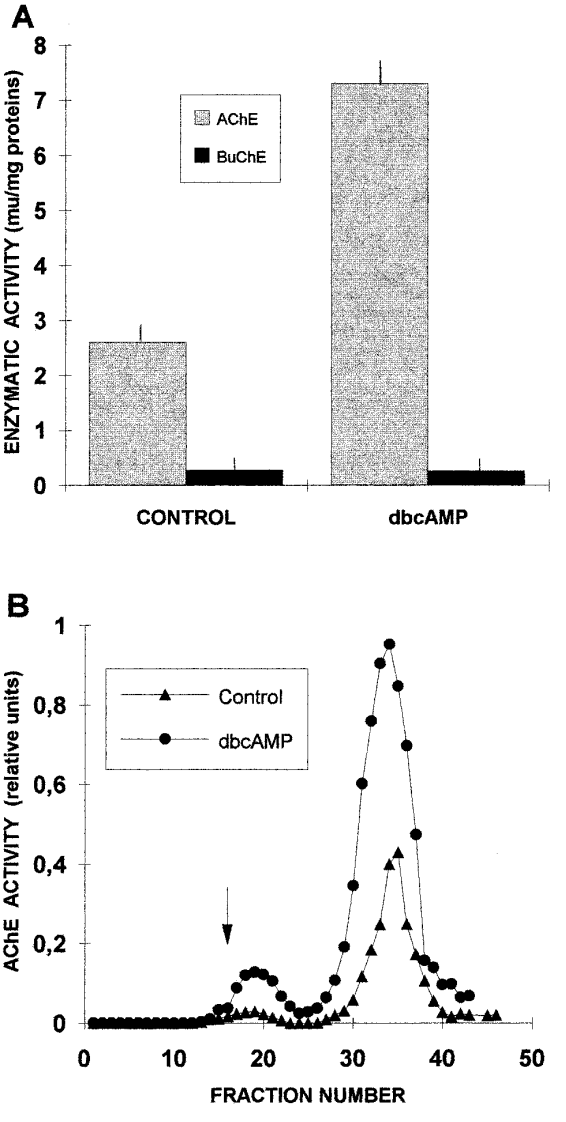

FIG. 4. AChE and BuChE activities in differentiated Neuro-2a cells. Neuroblastoma cells were grown as described under Materials and Methods and seeded at an initial density of 18×10^3 cells/cm^2. Then the cells were grown for 1 day, and on the second day and for the following 2 days the cells were incubated in the presence and the absence of 5 m*M* dbcAMP. (A) AChE and BuChE activity levels in control and differentiated cell extracts. Results are expressed as the mean ± SEM of three experiments. (B) Sucrose gradient sedimentation of AChE molecular forms in control and differentiated Neuro-2a cell extracts. The sedimentation marker corresponds to catalase (11.3 S).

polypeptide pattern as evidenced by SDS–PAGE (Fig. 5C). The increase in the level of the 35-kDa APP fragment observed at high cell confluence is probably due to an increase in APP processing rather than to an increase in APP expression, because the total amount of whole-length APP does not change with greater levels of cellular confluence (Fig. 6).

Neuro-2a cells seeded at an initial density of 18×10^3

cells/cm^2 and made to differentiate with 5 m*M* dbcAMP exhibited an approximately two-fold increase in the 35-kDa fragment compared to control. Under these same conditions, however, the levels of the 17-kDa fragment were not altered (Fig. 7).

DISCUSSION

The present report indicates that a 35-kDa amyloidogenic fragment of APP and the major molecular forms of AChE in Neuro-2a cells in culture are influenced by the extent of cellular confluence and by dbcAMP-induced differentiation. To our knowledge this is the first demonstration that a selective Aβ-bearing fragment of APP is subject to developmental regulation. The fact that both this APP fragment and AChE qualitatively respond in a similar manner to the same development stimuli points to a potential relationship between the mechanisms underlying their formation, a notion that is entirely consistent with the likely role(s) played by AChE in the processing of APP [35].

Current data indicate that AChE is implicated in neurodegenerative disorders such as AD as well as in the mechanism underlying neuronal differentiation [11–15]. As regards the latter, it has been reported that during neural development *in vivo* the characteristic changes observed in AChE activity correlate well with the general process of synaptogenesis [36, 37]. The present work further shows that in Neuro-2a cells *in vitro*, both the state of cellular confluence and neuronal differentiation influence total AChE activity, as well as the G_1 and G_4 molecular forms of the enzyme. These finding indicate that the aforementioned correlation between AChE and synaptogenesis may be related not only to the effects of cellular contact, but also to those involving various degrees of neuronal outgrowth. By contrast, the activity of BuChE, an enzyme that unlike AChE is not apparently involved in cholinergic synaptic transmission and shows a tendency to decrease during synaptogenesis [36, 37], was not affected by the foregoing developmental phenomena.

A behavior qualitatively similar to that shown by AChE in the Neuro-2a cells was exhibited by the 35-kDa Aβ-bearing fragment of APP, suggesting that this fragment could also serve a physiological function(s) during neuronal development. On the other hand, the 17-kDa fragment was not influenced by cellular contact or neuronal differentiation, thus implying that its regulation probably occurs through an alternative route which may not involve a transduction system identical to that controlling the production of the 35-kDa fragment.

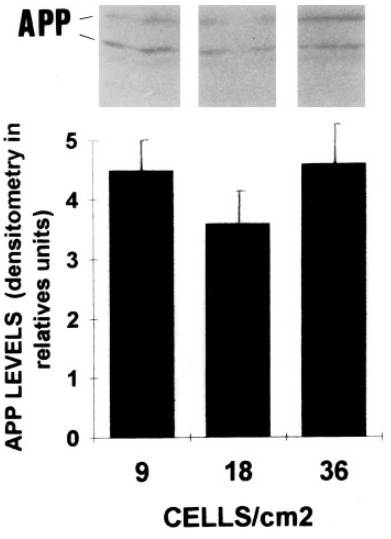

FIG. 6. APP levels in Neuro-2a cells grown at different states confluence. Cells were incubated as described in Fig. 5. Protein cell extracts (60 μg each) were separated in 16% Tris–tricine SDS–PAGE and immunoblotted using an antibody directed against residues 175–186 of the amino terminal of the APP molecule. Densitometric evaluation of immunoblots shown in the figure. Data are expressed as the mean ± SD of three separate experiments and values are normalized for the initial seeding density of 18 × 10^3 cells/cm^2.

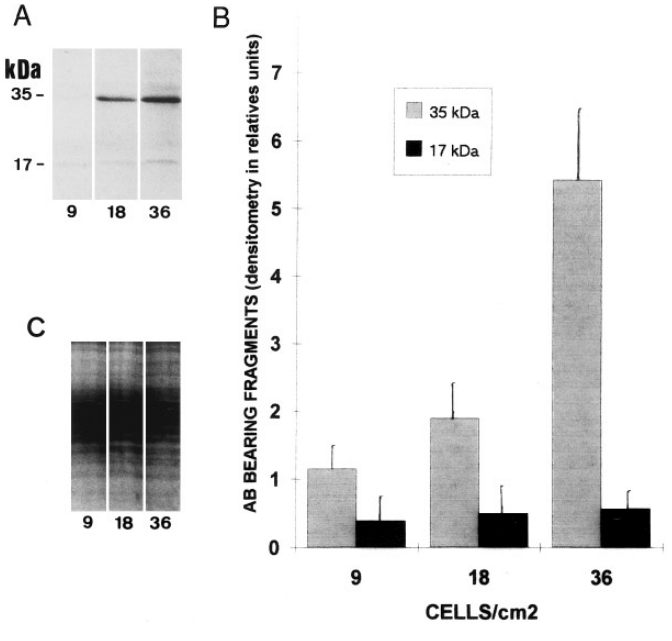

FIG. 5. Levels of APP fragments presents in Neuro-2a cells grown at different states of cell confluence. Cells were incubated as described in Fig. 3 at initial seeding densities of 9 × 10^3 cells/cm^2, 18 × 10^3 cells/cm^2, and 36 × 10^3 cells/cm^2. (A) Protein cell extracts (60 μg each) were separated in 16% Tris–tricine SDS–PAGE and immunoblotted using an antibody directed against the Aβ peptide (SGY2134). (B) Densitometric evaluation of immunoblots shown in (A). Data are expressed as the mean ± SEM of four separate experiments and values are normalized for the initial seeding density of 18 × 10^3 cells/cm^2. (C) Protein cell extracts (60 μg each) were separated in 16% Tris–tricine SDS–PAGE and stained with Coomassie blue.

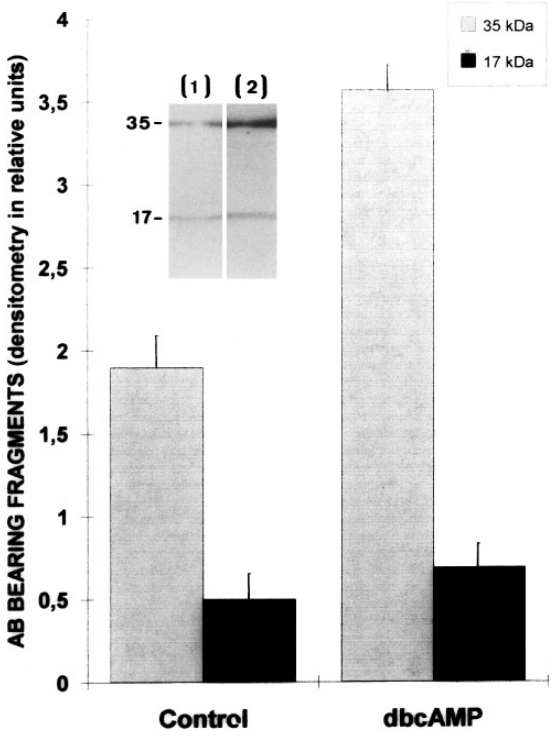

FIG. 7. Levels of APP fragments in differentiated Neuro-2a cells initially seeded at a cell density of 18×10^3 cells/cm^2 and grown in the presence and the absence of dbcAMP as described in the legends to Figs. 2 and 4. Protein cell extracts (60 μg each) were separated in 16% Tris–tricine SDS–PAGE and immunoblotted using an antibody directed against the Aβ peptide (SGY2134). Results of the densitometric evaluation of the blots (see inset) are expressed as the mean ± SEM of three separate experiments and values are standardized relative to those of control (relative units).

A 22-kDa fragment of APP bearing the Aβ peptide has been proposed as an intermediary derivative in the APP processing pathway which generates Aβ in both transfected human 293 cells [38] and in leupeptin-treated cells [39] and has also been described as a stable fragment containing the Aβ peptide in meningeal microvessels and other human regions [40]. Previous studies in our laboratory have shown that several ECM components, including laminin and collagen, influence the level of an amyloidogenic fragment of APP of 22 kDa in 3T3 fibroblasts [25]. Similar matrix components also affect a 35-kDa APP fragment in neuronal Neuro-2a cells [25], suggesting that this fragment can be a precursor of Aβ in the amyloidogenic APP processing pathway. In this context, it is interesting to mention that the Aβ peptide has been found associated with the ECM, where its operates as a neurite-outgrowth promoting factor [41].

Another aspect of this work involves the induction of neuronal differentiation by dbcAMP, a permeable analog of cAMP [24], and the consequent enhanced production of a selective Aβ-containing APP fragment. In this regard, it is known that the activation of protein kinases C and A regulate the production of the Aβ peptide, i.e., through stimulating the formation of trans-Golgi constitutive secretory vesicles that contain and transport intact APP [42–44]. It is therefore possible that the cAMP transduction system plays some role(s) in the developmental regulation of APP metabolism, perhaps by shifting the balance toward the amyloidogenic pathway. Although more work is required to resolve this point it is interesting to note that presenilins I and II, the products of two genes (located in chromosomes 14 and 1) linked to hereditary forms of AD, comprise proteins with various transmembrane segments which are homologues of receptor molecules associated with signal transduction mechanisms [45, 46].

Very recently the Aβ levels were studied in cultured postmitotic CNS neuronal cells (NT2N) produced by the terminal differentiation of a teratocarcinoma cell line (NT2) with retinoic acid [22]. In this system, APP processing is altered as NT2N cells age in culture such that the production of secreted $A\beta_{1-40}$ and $A\beta_{1-42}$ is dramatically increased, without a change in APP synthesis [22].

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REFERENCES

1. Soto, C., Brañes, C., Alvarez, J., and Inestrosa, N. C. (1994) *J. Neurochem.* **63,** 1191–1198.
2. Soto, C., Castaño, E., Frangione, B., and Inestrosa, N. C. (1995) *J. Biol. Chem.* **270,** 3063–3067.
3. Alvarez, J., Moreno, R. D., and Inestrosa, N. C. (1995) *Eur. J. Neurosci.* **7,** 152–159.
4. Alvarez, J., Moreno, R. D., Llanos, O., Inestrosa, N. C., Brandan, E., Colby, T., and Esch, F. S. (1992) *Neurosci. Lett.* **144,** 130–134.
5. Brandan, E., and Inestrosa, N. C. (1993) *Gen. Pharmacol.* **24,** 1063–1068.
6. Estus, S., Golde, T., Blades, D., Lowery, D., Eisen, M., Usiak, M., Qu, X., Tabira, T., Greenberg, B., Younkin, S. (1992) *Science* **255,** 249–253.
7. Higaki, J., Quon, D., Zhong, Z., and Cordell, B. (1995) *Neuron* **14,** 651–659.
8. Inestrosa, N. C., and Perelman, A. (1990) *J. Membr. Biol.* **188,** 1–9.
9. Taylor, P., and Radic, Z. (1994) *Annu. Rev. Pharmacol. Toxicol.* **34,** 281–320.
10. Fernández, H. L., Moreno, R., and Inestrosa, N. C. (1996) *J. Neurochem.* **66,** 1335–1346.
11. Arendt, T., Brückner, M. K., Lange, M., and Bigl, V. (1992) *Neurochem. Int.* **21,** 381–396.
12. Morán, M. A., Mufson, E. J., and Gómez-Ramos, P. (1993) *Acta Neuropathol.* **85,** 362–369.

13. Alvarez, A., Bronfman, F., Pérez, C. A., Vicente, M., Garrido, J., and Inestrosa, N. C. (1995) *Neurosci. Lett.* **201,** 49–52.
14. Inestrosa, N. C., Alvarez, A., Pérez, C. A., Moreno, R. D., Vicente, M., Linker, C., Casanueva, O. I., Soto, C., and Garrido, J. (1996) *Neuron* **16,** 881–891.
15. Smith, A. D., and Cuello, A. C. (1984) *Lancet* **I,** 513.
16. Shen, Z. X. (1994) *Med. Hypoth.* **43,** 21–30.
17. Breen, K. C. (1995) *Amyloid: Int. J. Exp. Clin. Invest.* **2,** 17–21.
18. Inestrosa, N. C., Moreno, R. D., and Fuentes, M.-E. (1994) *Neurosci. Lett.* **173,** 155–158.
19. Coleman, B. A., and Taylor, P. (1996) *J. Biol. Chem.* **271,** 4410–4416.
20. Hung, A. Y., Koo, E. H., Haass, C., and Selkoe, D. J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 9439–9443.
21. Pangalos, M. N., Shioi, J., and Robakis, N. K. (1995) *J. Neurochem.* **65,** 762–768.
22. Turner, R. S., Suzuki, N., Chyung, S. C., Younkin, S. G., and Lee, V. M.-Y. (1996) *J. Biol. Chem.* **271,** 8966–8970.
23. Blume, A., Gilbert, F., Wilson, S., Farber, J., Rosenberg, R., and Nirenberg, M. (1970) *Proc. Natl. Acad. Sci. USA* **67,** 786–792.
24. Prasad, K. N. (1975) *Biol. Rev.* **50,** 120–265.
25. Bronfman, F. C., Soto, C., Tapia, L., Tapia, V., and Inestrosa, N. C. (1996) *J. Cell. Physiol.* **166,** 360–369.
26. Lowry, D. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
27. Ellman, G. L., Courtney, K. D., Andres, V., Jr., and Featherstone, R. M. (1961) *Biochem. Pharmacol.* **7,** 88–95.
28. Inestrosa, N. C., Miller, J. B., Silberstein, L., Ziskind-Conhaim, L., and Hall, Z. W. (1983) *Exp. Cell Res.* **147,** 393–406.
29. Inestrosa, N. C., Fuentes, M. E., Anglister, L., Futerman, A. H., and Silman, I. (1988) *Neurosci. Lett.* **90,** 186–190.
30. Schagger, H., and van Jagow, G. (1987) *Anal. Biochem.* **166,** 368–379.
31. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
32. Shoji, M., Golde, T. E., Ghiso, J., Cheung, T. T., Estus, S., Shaffer, L. M., Cai, X.-D., McKay, D. M., Tinter, R., Frangione, B., and Younkin, S. G. (1992) *Science* **258,** 126–129.
33. Cole, G., Masliah, E., Huynh, T., de Teresa, R., Terry, R., Okuda, C., and Saitoh, T. (1989) *Neurosci. Lett.* **100,** 340–346.
34. Stieger, S., Bütikofer, P., Wiesmann, U. N., and Brodbeck, U. (1989) *J. Neurochem.* **52,** 1188–1196.
35. Campos, E. O., and Inestrosa, N. C. (1996) *Annu. Meet. Cell Biol. Soc. Chile,* p. 18. [Abstr. 34]
36. Layer, P. G., Alber, R., and Sporns, O. (1987) *J. Neurochem.* **49,** 175–182.
37. Layer, P. G., and Sporns, O. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 284–288.
38. Knops, J., Lieberburg, Y., and Sinha, S. (1992) *J. Biol. Chem.* **267,** 16022–16024.
39. Haass, C., Koo, E. H., Mellon, A., Hung, A. Y., and Selkoe, D. J. (1992) *Nature* **357,** 500–503.
40. Tamoaka, A., Kalaria, R. N., Lieberburg, Y., and Selkoe, D. J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 1345–1349.
41. Koo, E. D., Park, L., and Selkoe, D. J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 4748–4752.
42. Buxbaum, J. D., Koo, E. H., and Greengard, P. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9195–9198.
43. Xu, H., Greengard, P., and Gandy, S. (1995) *J. Biol. Chem.* **270,** 23243–23245.
44. Xu, H., Sweeney, D., Greengard, P., and Gandy, S. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 4081–4084.
45. Sherrington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., Ikeda, M., Chi, H., Lin, G., Holman, K., Tsuda, T., Mar, L., Foncin, J. F., Bruni, A. C., Montesi, M. P., Sorbi, S., Rainero, I., Pinessi, L., Nee, L., Chumakov, I., Pollen, D., Brookes, A., Sanseau, P., Polinsky, R. J., Wasco, W., Da Silva, H. A. R., Haines, J. L., Pericak-Vance, M. A., Tanzi, R. E., Roses, A. D., Fraser, P. E., Rommens, J. M., and George-Hyslop, P. H. St. (1995) *Nature* **375,** 754–760.
46. Levy-Lahad, E., Wasco, W., Poorkaj, P., Pettingell, W., Yu, C., Jondro, P., Schmidt, S., Wang, K., Crowley, A., Fu, Y., Guenette, S., Galas, D., Nemens, E., Wijsman, E., Bird, T., Schellenberg, G., and Tanzi, R. (1995) *Science* **269,** 973–977.

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