Alzheimer’s β-Amyloid, Human Islet Amylin, and Prion Protein Fragment Evoke Intracellular Free Calcium Elevations by a Common Mechanism in a Hypothalamic GnRH Neuronal Cell Line*

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A growing number of reports suggest that elevated levels of extracellular Alzheimer’s β-amyloid protein alter the homeostasis of free [Ca\(^{2+}\)]\(_i\), in different cell types of the mammalian brain. In line with these results, we have previously shown that AβP[1–40] forms cation-selective channels (Ca\(^{2+}\)-selective) across artificial planar bilayers formed from acidic phospholipids and across excised membrane patches from immortalized hypothalamic GnRH neurons (GT1-7 cells), suggesting that the nonregulated Ca\(^{2+}\)-influx through these spontaneously formed “amyloid channels” may provide a mechanism to explain its toxicity (1). We have now found and report here that the application of AβP[1–40] to GT1-7 neurons consistently elevates [Ca\(^{2+}\)]\(_i\), levels. We also found that human islet amylin and the prion protein fragment (PrP106–126), peptides that acquire β-pleated sheet conformation in water solutions and have been reported to form ion channels across planar bilayer membranes, also increase cytosolic free calcium in GT1-7 neurons. Searching for protective agents, we found that soluble cholesterol, known to decrease the fluidity of the cell membrane, inhibits AβP[1–40]-evoked [Ca\(^{2+}\)]\(_i\), rise. These results suggest that unregulated Ca\(^{2+}\) entry across amyloid channels may be a common mechanism causing cell death, not only in diseases of the third age, including Alzheimer’s disease and type 2 diabetes mellitus, but also in prion-induced diseases.

Alzheimer's disease (AD) is a dementia characterized by the loss of mental and physical functions and the presence in the brain of protein deposits called extracellular plaques and intracellular aggregates called fibril tangles. The principal constituents of these deposits are peptides, 39–43 residues long, termed amyloid β-proteins (AβP), that exhibit distinct domains that acquire β-pleated sheet structure and α-helical architecture. These peptides originate from the proteolytic degradation by secretases of the amyloid precursor protein defined by a locus on chromosome 21 (4, 5). It is well established that β-amyloid peptides in water solutions acquire β-pleated sheet architecture and form insoluble aggregates. Earlier studies showed that application of AβP[1–40] to either primary cultures of rat hippocampal neurons (6) or hippocampal slice cultures (7) had toxic effects. These results provide support for the idea that AβP[1–40] might be the causal agent of neuronal death in AD (8). Interestingly, islets of Langerhans of patients with non-insulin-dependent diabetes mellitus, which like AD is an age-dependent disease, also exhibit protein deposits. The major constituent of the pancreatic islet plaques is an amyloid peptide termed amylin (9). It has also been reported that human islet amylin (37-amino acid residues), found in protein deposits within islets of Langerhans of patients with non-insulin-dependent diabetes mellitus, is toxic to both cultured islet cells (10) and rat hippocampal neurons (11) and also induces apoptosis in rat cortical neurons (12). Although there is ~93.5% sequence homology between human and rat amylin, the latter peptide was found to be without effect on pancreatic islet cells. As to the mechanism by which amylin is toxic, it has been recently shown that human amylin forms ion channels across planar lipid bilayer membranes, but rat amylin does not (13). Furthermore, we have shown that AβP[1–40] also form cation channels across planar bilayers formed from negatively charged phospholipids (14–16). In addition, other results (17, 18) suggest that amylin and toxicity may involve the formation of unregulated cation channels across the membrane of susceptible cells.

Recent reports support the idea that amyloid peptides play a key role in cell death in pancreatic islet β-cell (10, 13) and in neuronal death (1, 11, 12). Because AβP peptides are released to the extracellular space of the brain of normal subjects (19), it remains to be established which factors are expressed in the senile brain that transform specific neurons and make them susceptible to amyloid β-protein. Furthermore, owing to the fact that amylin is co-secreted together with insulin in normal islets (9), we may also ask what makes the pancreatic β-cell susceptible to amylin.

As the search for the cause(s) of AD progresses, it has become evident that more than one factor is responsible for each modality of AD. Gene mutations account for changes in the phenotype of specific proteins linked with a modality of AD. It has been suggested that β-amyloid peptides may induce oxidative stress altering catecholamine metabolism in the brain. Fraser et al. (20) and Matson et al. (21) had already proposed that β-amyloid peptides destabilize Ca\(^{2+}\) homeostasis in neurons. Although the mechanism by which specific brain cells can become susceptible to free AβP peptides remains to be elucidated, we suggested that the ability of AβP[1–40] to form unregulated cation-selective channels across the plasma mem-

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†The abbreviations used are: AD, Alzheimer’s disease; AβP, amyloid β-protein.

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brane of susceptible cells could explain amyloid toxicity (14–16). In support of this model, we showed that (8) PrP[1–40] also forms cation-selective channels across excised membrane patches from immortalized hypothyamic GnRH neurons (1) in accord with other studies on a different neuronal tissue (20, 21). A distinctive feature of AβP peptides in water solution is their ability to acquire β-sheet architecture, a conformation that is crucial for the formation of cation-selective channels (22, 23).

In line with the cation channel hypothesis, we now show that not only brain β-amylloid and islet amylin but also prion peptides induce substantial [Ca^{2+}] i elevations in GT1-7 neuronal cell line. Prion peptides originate from the conversion of cellular prion protein (PrP^C) to the pathogenic form (PrP^Sc) and are associated with several diseases, including scrapie, Kuru disease, Creutzfeldt-Jakob disease, and bovine spongiform encephalopathy (23). In a previous study, Forloni et al. (24) showed that PrP106–126 induces cell death in primary cultures of rat hippocampal neurons. Furthermore, it has been recently shown that a 21-amino acid synthetic fragment of the prion protein PrP106–126, which has no homology in its sequence with those of AβP[1–40] and amylin, exhibits β-sheet structure in water and also forms ion channels across planar lipid bilayer membranes (25).

In this study, we compare the effects of different amyloid peptides on the intracellular free calcium concentration of GT1-7 hypothalamic neuronal cell line (26, 27). For this task we used a high resolution, multi-site video imaging system with fura-2 as cytosolic free calcium ([Ca^{2+}] i) reporter fluorescence probe (27–29). We found and report here that GT1-7 neurons (30) exposed to AβP[1–40], human islet amylin, or PrP106–126 exhibited marked elevations in [Ca^{2+}] i levels. Furthermore, we noted that not all the cells exposed were affected by these peptides. These results provide persuasive evidence supporting the idea that the spontaneous interactions between amyloid peptides and the plasma membrane of susceptible cells lead to the formation of unregulated Ca^{2+} channel (13–16).

In an effort to find the means of protecting target cells from the toxic effects of active peptides used in this study, we selected AβP[1–40] and compared its effects on [Ca^{2+}] i in GT1-7 cells untreated (control) and cells pretreated with soluble cholesterol, known to interact with the sphingolipid-rich external leaflet of the cell plasma membrane (31–34). These interactions between cholesterol and sphingomyelin should bring about a substantial decrease in the fraction of acidic phospholipids in the external leaflet (33–35). We found and report here that soluble cholesterol is an effective inhibitor of AβP[1–40]-induced free calcium elevation in GT1-7 cells.

**EXPERIMENTAL PROCEDURES**

**Intracellular Free Calcium Measurements—**GT1-7 immortalized hypothalamic neurons (provided by Dr. R. Weiner, University of California at San Francisco) were cultured as described previously (26, 30). Briefly, GT1-7 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 5% calf serum and 5% horse serum. After enzymatic dissociation, cells were resuspended in serum-free Dulbecco’s modified Eagle’s medium. Cells were plated on glass coverslips coated with a mixture of 0.1% polyethylenimine and 0.01% laminin at a concentration of 1 × 10^5 cells/cm^2. After a few days in culture the medium was replaced by basal salt solution (130 mM NaCl, 5.5 mM glucose, 5.4 mM KCl, 1.8 mM CaCl2, 20 mM NaHepes, pH 7.4) containing 1.5 μM fura-2AM (acetoxymethylester cell-permeant form, Molecular Probes, Eugene, Oregon). Cells were incubated in the presence of fura-2AM at 37°C. After a loading period (60 min), cells were observed using an inverted fluorescence microscope (Nikon, Japan) equipped with a high resolution video camera (Hamamatsu Photonics Co., C-2400, Japan). Fura-2 [Ca^{2+}] i images were recorded at a rate of 30 images/s on VCR tapes. Stored images were analyzed using an ad hoc hardware and software system controlled by a computer (PC-300, Mitsubishi Kasei).

Each image represents the fluorescence intensity from a rectangular optical field (360 × 420 μm²). The system allowed us to record the ratio of the intensity emitted at 510 nm from pixels of images obtained alternating the excitation wavelength between 340 and 360 nm by means of a rotating filter wheel. The optical field could monitor from 50 to 100 cells simultaneously. Each ratio was automatically converted into [Ca^{2+}] i using a calibration curve (ratio versus [Ca^{2+}] i) stored in the hard disc of the PC.

As a rule, prior to the addition of a specific peptide to the solution bathing the cells, we recorded the resting levels of neuronal [Ca^{2+}] i, during a 5-min period. All records were made at 37°C. The statistical Analysis—Our system enabled us to accurately measure both the amplitude of early peak [Ca^{2+}] i rise and the latency of the response. To form histograms with these data, we grouped the cell responses in bins (bin width, 10 μM), and then we plotted the number of cells/bin as a function of peak [Ca^{2+}] i values. To obtain the statistical distribution of the early peak [Ca^{2+}] i rise, we fitted a normal probability curve to the data, i.e. number of events/bin as a function of the amplitude of the early peak [Ca^{2+}] i rise. As to the statistical distribution of the latency, i.e. the time to the first discernable increase in [Ca^{2+}] i, level following the addition of a given peptide (termed “[Ca^{2+}] i rise latency” in the figures), we opted for a Poisson distribution of the data, i.e. number of events per bin as a function of the latency of the peak [Ca^{2+}] i rise. For this task we used a least squares regression algorithm provided by a software package Origin 5.0 (Microcal, Northampton, MA). The probability values (0–100%) were obtained from the fit (mean ± standard deviation). Latencies were measured on the same cell population.

**Chemicals and Reagents—** AβP[1–40], AβP[40–1], human amylin, rat amylin, and PrP106–126 were obtained from Bachem F.A.G. (Bubendorf, Switzerland) and dissolved in distilled water at a concentration of 0.2 mM and stored at –80°C. During the experiments we added aliquots from the peptide stock solution directly to the solution (~0.5 ml) bathing the cells.

**Cell Viability Assay—** GT1-7 cells were plated on 96-well culture dishes at a concentration of 2 × 10^3 cells/cm² and maintained in culture for 2 days. After one day of exposure to AβP[1–40], we examined the GT1-7 neuron using a viability kit (available from Dojindo Chemicals, Japan). The assay measures the mitochondrial-dependent conversion of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium monosodium salt to water-soluble formazan (27).

Although fluorescent reporter dyes for intracellular free calcium and cell viability are available and, at least in principle, they could be used to monitor simultaneously both [Ca^{2+}] i and cell viability, we found that the bleaching (excitation at 380 nm) of fura-2 was ~30 min for fluorescence intensity (excitation at 380 nm and 350 nm). By contrast, the half-time for the viability test was ~180 min. These long lasting exposures to UV light are per se cytotoxic, making it impossible to distinguish between cell death caused by elevated [Ca^{2+}] i, and that caused by the UV light. Therefore, [Ca^{2+}] i and cell viability were monitored on two different groups of cells from the same cell culture.

**Cholesterol Treatment—** Water-soluble cholesterol (polyoxyethylene cholesterol succinate, Sigma) was dissolved in distilled water at a concentration of 10 mg/ml. Water-soluble cholesterol was added to the medium bathing the cells (final concentration, ~0.52 μM) 60 min prior to the initiation of the [Ca^{2+}] i imaging experiment. At the end of a 60-min period, the medium was removed from both the dish with GT1-7 cells exposed to cholesterol and the dish with control GT1-7 cells. Immediately after, both dishes were washed with fresh cholesterol-free medium. This procedure was repeated three consecutive times. Thereafter, we carried out the protocol designed to test the effects of AβP[1–40].

**RESULTS**

To study the interactions between endogenous peptides of the amyloid family and the plasma membrane of susceptible neuronal cells, we monitored the levels of [Ca^{2+}] i prior to and after the application of a given peptide. To this end, we carried out comparative studies of the [Ca^{2+}] i changes induced by active peptides in GT1-7 neurons. For this purpose, we used a Ca^{2+} imaging system based on fura-2 as an intracellular [Ca^{2+}] i reporter and a high resolution video camera to capture the emission from fura-2 acid trapped inside the cells. Other laboratories have already used this technique to monitor synaptic activity in primary cultures of rat neurons (28, 29). In addition to the high resolution, the video camera allows the
simultaneous detection of the [Ca\(^{2+}\)] changes in a population of GT1-7 cells (50–100 cells/optical field), facilitating the statistical analysis.

**Effects of A\(\beta\)[1–40], Human Islet Amylin, and Prion Peptide on Intracellular Free Calcium in GT1-7 Cells**—Fig. 1 shows the effects of A\(\beta\)[1–40] (Fig. 1A, line a), human amylin (Fig. 1A, line b), and prion peptide PrP\(_{106–126}\) (Fig. 1A, line c) on the time course of intracellular free calcium in GT1-7 cells (Fig. 1A), maximum [Ca\(^{2+}\)] levels (Fig. 1B), and the percentage of GT1-7 cells responding to the peptides (Fig. 1C). The records shown in Fig. 1A were aligned to the time at which the peptides were applied (indicated by the arrow). Fig. 1A shows the time course of [Ca\(^{2+}\)], before and after the application of 10 \(\mu\)M of either A\(\beta\)[1–40] (Fig. 1A, line a), A\(\beta\)P[40–1] (Fig. 1A, line b), human amylin (Fig. 1A, line c), rat amylin (Fig. 1A, line d), or PrP\(_{106–126}\) (Fig. 1A, line e). The peptides that did not elicit measurable changes in [Ca\(^{2+}\)], namely, A\(\beta\)P[40–1] and rat amylin, were considered to be inactive and, for this reason, were taken as a control. The effects of different peptides (including active and inactive peptides) on intracellular free calcium were further evaluated comparing both the size of the peak [Ca\(^{2+}\)], rise over basal (Fig. 1B) and the fraction of cells responding per optical field (Fig. 1C).

The time course of the [Ca\(^{2+}\)], rise evoked by 10 \(\mu\)M of active amyloid peptides (Fig. 1A, lines a, c, and e) can be compared with that of inactive peptides (Fig. 1A, lines b and d). It is clear from Fig. 1A (line a) that the [Ca\(^{2+}\)] rise consists of an early transitory and a delayed sustained elevation of [Ca\(^{2+}\)]. A significant fraction of the cells responded to each active peptide with an elevation of [Ca\(^{2+}\)], characterized by a biphasic rise, i.e., an early peak followed by a sustained level. The peptide with the reverse sequence of Alzheimer’s A\(\beta\)[1–40] was without effect (Fig. 1A, line b). For this reason we selected A\(\beta\)P[40–1] as a control for possible nonspecific effects A\(\beta\)[1–40] on [Ca\(^{2+}\)]. Fig. 1B shows the average value for the amplitude of the rise in [Ca\(^{2+}\)], over basal (~100 nM) induced by each peptide together with the corresponding controls. The mean value of each percentage of cells responding is shown in Fig. 1C. From Fig. 1B it is apparent that the peak [Ca\(^{2+}\)], rise evoked by 10 \(\mu\)M A\(\beta\)P[1–40] (183 ± 7 nM over basal, mean ± S.E., \(n = 250\) cells) is significantly greater than that induced by human amylin (138 ± 11 nM, \(n = 200\)), which is greater than that induced by the prion PrP\(_{106–126}\) (98 ± 11 nM, \(n = 250\)). Fig. 1C illustrates that the percentage of GT1-7 cells responding follows a similar trend with A\(\beta\)P[1–40], affecting more cells than human amylin or prion peptides. We also compared the time lapsed between the addition of the peptide and the maximum level of [Ca\(^{2+}\)] for each active peptide. The mean value of the time to peak was 13 ± 1 s (mean ± S.E., \(n = 250\)) for 10 \(\mu\)M A\(\beta\)P[1–40], 23 ± 3 s (\(n = 200\)) for 10 \(\mu\)M human amylin, and 82 ± 6 s (\(n = 250\)) for 10 \(\mu\)M PrP\(_{106–126}\) peptide.

**A\(\beta\)P[1–40] Dose Response Analysis**—The A\(\beta\)P[1–40] dose response diagrams depicted in Fig. 2A represent the fraction of cells (in percentages) that responded with a characteristic [Ca\(^{2+}\)] elevation to increasing concentrations of A\(\beta\)P[1–40]. At 10 \(\mu\)M A\(\beta\)P[1–40], 77 ± 5% of the GT1-7 cells responded (filled circles; mean ± S.E., \(n = 6\)). Fig. 2B shows peak [Ca\(^{2+}\)] elevation over basal. The average peak rise in [Ca\(^{2+}\)], induced by 10 \(\mu\)M A\(\beta\)P (1–40) was 150 ± 6 nM (open circles; mean ± S.E., \(n = 300\)). In Fig. 2C is the latency of the peak [Ca\(^{2+}\)] rise. As expected, the latency in the onset of a measurable [Ca\(^{2+}\)] rise shortened as the peptide dose was increased. Fig. 2D shows GT1-7 neuronal viability, determined as described under “Experimental Procedures,” decreased with A\(\beta\)P[1–40] concentration. It is clear that the fraction of cells responding to A\(\beta\)P[1–40] and the size of the peak [Ca\(^{2+}\)], over basal induced by A\(\beta\)P[1–40] increases in a dose-dependent manner. As expected, the latency in the onset of a measurable [Ca\(^{2+}\)], rise shortened as the peptide dose was increased (Fig. 2C). It should be mentioned here that after 24 h of exposure to 10 \(\mu\)M A\(\beta\)P[1–40], we observed cellular apoptosis in 50% of the GT1-7 cells (Fig. 2D).

**Comparative Statistical Analysis of the [Ca\(^{2+}\)] Changes Evoked by Different Active Amyloid Peptides**—Fig. 3 shows six histograms constructed from measurements of the amplitude of the early peak [Ca\(^{2+}\)], elevation for each cell on the optical field (panels on the left) and the time lapsed between the addition of the specific peptide (10 \(\mu\)M for all the experiments) and the start of the first phase (latency) of the elevation of [Ca\(^{2+}\)], (panels on the right). In all the panels, the vertical axes represent the number of cells responding to the peptide divided by the total number of cells examined (in percentages). For the panels on the left, the horizontal axes represent the amplitude of the early peak [Ca\(^{2+}\)], elevation (bin = 10 nM). For the panels on the right, the abscissa represents the latency of the response (bin = 5 s). All other experimental procedures being the same, this comparative analysis suggests that A\(\beta\)P[1–40] is indeed more effective in promoting increasing the [Ca\(^{2+}\)], than the other active peptides, namely human amylin and the prion PrP\(_{106–126}\).

**GT1-7 Cell-to-Cell Variations in the [Ca\(^{2+}\)] Response to Either A\(\beta\)P[1–40] or Human Amylin**—Fig. 4 depicts 50 records of the time course of the [Ca\(^{2+}\)], elevations in response to 10 \(\mu\)M A\(\beta\)P[1–40] from randomly selected cells from the optical field. The arrows above the traces indicate the time at which the chamber was superfused with medium containing 10 \(\mu\)M A\(\beta\)P[1–40]. It may be seen that there is an important disparity in the responses. The amplitude of the peak [Ca\(^{2+}\)], rise and the type of response varied, some cells exhibit [Ca\(^{2+}\)], oscillations (cells 25, 27, 29, 30, 34, 37, 42, and 50), and others exhibit biphasic [Ca\(^{2+}\)], elevations (cells 1, 2, 4, 7, 10, 14, 16–18, 20, 22, 35, and 46). Alignment of the records from 50 cells revealed
that not only did the time course of the \([\text{Ca}^{2+}]_i\) rise vary among the peptides used here, but also the response to the same peptide varied from cell to cell (Figs. 4 and 5).

Fig. 5 shows the time course of \([\text{Ca}^{2+}]_i\) in six cells, at different locations of the optical field, prior to and after the application of 50 \(\mu\)M human amylin. As shown in Fig. 5, soon after they were exposed to human amylin, GT1-7 cells shown with lines \(d\) and \(e\) responded with minute \([\text{Ca}^{2+}]_i\) oscillations. Records \(a\), \(b\), and \(d\) clearly show that the time course of the \([\text{Ca}^{2+}]_i\) response to the pancreatic islet amyloid peptide varies among GT1-7 cells.

Although we have no explanation for the variation in the \([\text{Ca}^{2+}]_i\) response to either \(\text{A}\beta\text{P}[1–40]\) or human amylin in GT1-7 neurons, it is reasonable to assume that the lipid and protein composition of the target membrane determines the amyloid peptide susceptibility. We propose that the primary factor in cell susceptibility to amyloid peptides is the lipid composition of the external leaflet of the cell membrane.
Effects of Soluble Cholesterol on GT1-7 Neuronal Susceptibility to Amyloid—It is well established that cholesterol interacts with sphingolipids, which are abundant in the extracellular leaflet of cell membrane (33–36). These interactions alter the glycerophospholipid composition of cell plasma membrane leading to profound changes in membrane fluidity. We tested the ability of soluble cholesterol to protect target cells from amyloid peptide toxicity.

The effects of soluble cholesterol (ca. 0.52 mM) on \([Ca^{2+}]_i\) are shown in Fig. 6. The effects of 10 \(\mu M\) \(\text{A}\beta\text{P}[1–40]\) on \([Ca^{2+}]_i\) levels in control (Fig. 6A, left panel) and in soluble cholesterol-treated GT1-7 cells (Fig. 6A, right panel). Fig. 6B compares the percentages as a function of the size of the peak \([Ca^{2+}]_i\), rise over basal both in control cells (left side) and in cholesterol-treated cells (right panel). A comparison of the histograms shown in Fig. 6B, control cells (left panel) and cholesterol pretreated GT1-7 cells (right panel) reveals that soluble cholesterol inhibits \(\text{A}\beta\text{P}[1–40]\)-evoked \([Ca^{2+}]_i\), rise. Indeed, the average peak \([Ca^{2+}]_i\), rise over basal in control cells is 169 ± 6 nm (mean ± S.E., \(n = 300\)), whereas in cholesterol-treated GT1-7 cells it is only 83 ± 5 nm. Furthermore, Fig. 6C shows that soluble cholesterol increases the latency of the \([Ca^{2+}]_i\), response to 10 \(\mu M\) \(\text{A}\beta\text{P}[1–40]\). Fig. 6C shows that in control cells (left panel) the latency is approximately half (15 ± 1 s) of that in soluble cholesterol-treated GT1-7 cells (right panel; 27 ± 3 s).

**DISCUSSION**

We have shown here that the exposure of GT1-7 hypothalamic neurons to either Alzheimer’s \(\beta\)-amyloid protein or human pancreatic islet amylin can induce substantial increases in cytosolic free calcium concentration in GT1-7 cells. We also showed here that the pathogenic prion \(\text{PrP}106–126\) peptide is nearly as effective as \(\text{A}\beta\text{P}[1–40]\) evoking \([Ca^{2+}]_i\), rises. These results provide compelling evidence supporting the idea that amyloid \(\beta\) peptide, pancreatic islet amylin, and prion peptide spontaneously form cation channels across the plasma membrane of susceptible target cells. \(\text{A}\beta\text{P}[1–40]\) can induce marked increases in \([Ca^{2+}]_i\) resulting from increased \(Ca^{2+}\) influx through \(\text{A}\beta\text{P}\) channels spontaneously formed in the plasma membrane of susceptible GT1-7 neurons. This unregulated \(Ca^{2+}\) influx might eventually saturate intracellular \(Ca^{2+}\) stores, causing cell death.

**Cation Channel Hypothesis Provides a Mechanism to Explain Amyloid Toxicity**—Taken together the data presented here provide strong support for the “amyloid channel hypothesis” (13–16, 22, 37), which provides a molecular mechanism for cell degeneration in the brain of AD patients. One can extend this concept to explain cell toxicity of other amyloid peptides suspected to be causal factors of other age-related diseases.

In search for a common mechanism to explain channel formation (22), we compared the amino acid sequence alignment of the...
peptides used in this study: AβP[1–40], DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVY; AβP[40–1], VVG-GVMLHAGKNSGVDEAFFVLFQKHQHYVGSDDHFRK; human amylin, KCNTACCATQRLANFLVHSSNFLGAILSSATSNTVGSNTY; rat amylin, KCNTACCATQRLANFLVRSNNLLGPVLPHTNQSVSNTY; and PrP106–126, KTNKHMAGAAAGAVVGGLG. As shown, there is no sequence analogy between the peptides, namely Alzheimer’s β-amyloid, islet amylin, and prion peptide PrP106–126. It should be noted that although murine and human amylin exhibit 93.3% homology, paradoxically only human amylin is neurotoxic. From this sequence analysis we conclude that crucial structural domains of the free peptides in water solution must allow specific interactions between the external lipid leaflet of cell membrane and the peptide leading to the spontaneous pore formation (13–16). Indeed, the structural ability of the toxic peptides used here to acquire α-helical and β-pleated sheet architecture has been established by NMR analysis of crystals formed from highly purified recombinant prion peptides (23). Because the peptides used here share the ability to acquire β-pleated sheet structures and form α-helical domains in water solutions (22, 23), we concluded that peptide conformation in solution plays a crucial role in peptide binding to the membrane and channel architecture across the membrane (38, 39).

The structural transition from the cellular prion protein (PrPSc) that is rich in α-helices to the pathological form (PrPSc) that has a high β-sheet content seems to be the fundamental event underlying the prion diseases (23) plays a fundamental role in the ability of the peptide to spontaneously form cation channels across the plasma membrane of susceptible cells.

The GT1-7 Cell Line as a Neuronal Model System—The hypothalamic GT1-7 cell line used here retains several features of the hypothalamic GnRH neurons (30). Indeed, the cultured GT1-7 cells used in the present study extend neuritic processes, express neuronal marker proteins (microtubule-associated protein 2, termed MAP2, and neurofilaments) (16), express GnRH receptors, and exhibit t-type Ca2+ channels and Ca2+-activated, charibdotoxin-sensitive K+ channels of large conductance (32). As such the GT1-7 hypothalamic cell line is a valid neuronal model to study amyloid β peptide and islet amylin, peptides suspected to be involved as causal factors in Alzheimer’s disease and type 2 diabetes mellitus.

Heterogeneity in the GT1-7 cell [Ca2+]i Response to Amyloid Peptides: Requirement for the Expression of Susceptibility Factors?—One of the advantages of the [Ca2+]i imaging system used in this study is the ability to report the time course of [Ca2+]i changes in 50–100 GT1-7 neurons simultaneously. The time course of amyloid peptide-induced [Ca2+]i rise was used to advantage to evaluate the efficacy of potential protective agents, such as soluble cholesterol. Furthermore, this method enabled us to note that a fraction of the GT1-7 cells were more susceptible to the peptides than others and that a fraction of the cells were protected.

In an attempt to explain the diversity of responses, we carried out preliminary immunocytochemical characterization of GT1-7 cells cultured in the presence of AβP[1–40] (not shown here). We found that the neuronal marker MAP2 was homogeneously localized throughout cell bodies and dendritic trees of GT1-7 cells. In a previous study, we showed that the antibody to AβP was bound to restricted regions of the cell bodies and processes (16). Thus, it is possible that discrete membrane areas of the GT1-7 cells are more susceptible to different peptides, including AβP[1–40].

Soluble Cholesterol: A Potential Therapeutic Factor?—Lipids are now recognized not only as constituents of the fluid matrix of biological membranes but also are thought to play a role in the formation of microdomains in membranes. It is widely
accepted that the lipid bilayer of the plasma membrane serves as a solvent for membrane proteins. Cell membrane lipids consist mainly of three different classes of lipids, namely glycerolipids, sterols, and sphingolipids, the proportion of which depends on cell type (31, 32). Furthermore, glycerolipids are the most abundant class with ratios of phosphatidylcholine and phosphatidylethanolamine to total phospholipids of 30–60% (31). Cholesterol, which is the major sterol, is preferentially localized to the plasma membrane and amounts to 10–20% of the total plasma membrane lipid (32). Furthermore, in model membrane systems such as planar lipid bilayers and unilamellar vesicles (liposomes), cholesterol affinity for sphingomyelin is greater than that for glycerophospholipids (31). We also know that interactions of protein molecules and membrane lipids are strongly influenced by membrane fluidity. Cholesterol has been shown to decrease the fluidity of artificial and natural membranes affecting the ability of antibiotic peptides to form channels (33, 34). Coincidently, human amylin does not form channels across cholesterol-rich membranes (13, 35). Aβ[1–40] inserts itself across planar bilayer formed from acidic phospholipids but not across cholesterol bilayers. A recent paper reported that the fragment AβP (25–35) toxicity is inhibited by cholesterol in cultured PC12 cells (35).

Increasing the fraction of cholesterol molecules in the membrane may bring about a substantial decrease in the fraction of acidic phospholipids in the membrane, known to be important in neural membranes and further molecular rearrangements, leaving the ϕ-pleated sheet domains lining the pore of the putative amyloid cation channel. The formation of unregulated cation channels in susceptible cells contributes to metabolic stress and eventual cell death.

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Alzheimer's β-Amyloid, Human Islet Amylin, and Prion Protein Fragment Evoke Intracellular Free Calcium Elevations by a Common Mechanism in a Hypothalamic GnRH Neuronal Cell Line

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