Enhancement of 45Ca\(^{2+}\) Influx and Voltage-dependent Ca\(^{2+}\) Channel Activity by \(\beta\)-Amyloid-(1–40) in Rat Cortical Synaptosomes and Cultured Cortical Neurons

MODULATION BY THE PROINFLAMMATORY CYTOKINE INTERLEUKIN-1\(\beta\)

Aioife MacManus, Martin Ramsden‡, Michael Murray, Zaineb Henderson‡, Hugh A. Pearson‡, and Veronica A. Campbell§

From the Department of Physiology, Trinity College, Dublin 2, Ireland and §School of Biomedical Sciences, University of Leeds, Leeds LS2 9JT, United Kingdom

\(\beta\)-Amyloid protein is thought to underlie the neurodegeneration associated with Alzheimer’s disease by inducing Ca\(^{2+}\)-dependent apoptosis. Elevated neuronal expression of the proinflammatory cytokine interleukin-1\(\beta\) is an additional feature of neurodegeneration, and in this study we demonstrate that interleukin-1\(\beta\) modulates the effects of \(\beta\)-amyloid on Ca\(^{2+}\) homeostasis in the rat cortex. \(\beta\)-Amyloid-(1–40) (1 \(\mu\)M) caused a significant increase in 45Ca\(^{2+}\) influx into rat cortical synaptosomes via activation of L- and N-type voltage-dependent Ca\(^{2+}\) channels and also increased the amplitude of N- and P-type Ca\(^{2+}\) channel currents recorded from cultured cortical neurons. In contrast, interleukin-1\(\beta\) (5 ng/ml) reduced the 44Ca\(^{2+}\) influx into cortical synaptosomes and inhibited Ca\(^{2+}\) channel activity in cultured cortical neurons. Furthermore, the stimulatory effects of \(\beta\)-amyloid protein on Ca\(^{2+}\) influx were blocked following exposure to interleukin-1\(\beta\), suggesting that interleukin-1\(\beta\) may govern neuronal responses to \(\beta\)-amyloid by regulating Ca\(^{2+}\) homeostasis.

\(\beta\)-Amyloid (\(\beta\)(1–40)) is a peptide fragment derived from proteolytic processing of \(\beta\)-amyloid precursor protein (\(\beta\)APP) (1), which accumulates as an insoluble extracellular deposit around neurons, giving rise to the senile plaques associated with Alzheimer’s disease (AD) (2). Increased neuronal expression of the proinflammatory cytokine interleukin-1\(\beta\) (IL-1\(\beta\)) is an additional neuropathological hallmark of AD (3), and inflammatory mediators such as IL-1\(\beta\) may govern neuronal responses to \(\beta\)-amyloid by regulating Ca\(^{2+}\) homeostasis.

Understood. Neuronal apoptosis is the suspected causative factor of neurodegeneration in AD, and \(\beta\)A fragments have been shown to promote apoptosis in vitro in human-derived neurotypic cells (8) and cultured neurons (9). The mechanism underlying \(\beta\)A\(^{\pm}\)-induced apoptosis is thought to involve disregulation of Ca\(^{2+}\) homeostasis (10). In the C6 glial cell line, expression of the Ca\(^{2+}\)-binding protein calbindin was found to suppress \(\beta\)A\(^{\pm}\)-induced apoptosis (11), providing evidence for the involvement of Ca\(^{2+}\) fluxes in \(\beta\)A\(^{\pm}\)-induced apoptosis. In this study we report that \(\beta\)A-(1–40) (i) promotes a stimulation of \(45\)Ca\(^{2+}\) influx into cortical synaptosomes via activation of L- and N-type voltage-dependent Ca\(^{2+}\) channels (VDCCs) and (ii) increases the amplitude of N- and P-type VDCC current in cultured cortical neurons. Furthermore, the \(\beta\)A-(1–40)-induced increase in Ca\(^{2+}\) influx is blocked by the proinflammatory mediator IL-1\(\beta\) in both cortical synaptosome preparations and cultured cortical neuron preparations, providing evidence for a physiological interaction between IL-1\(\beta\) and \(\beta\)A-(1–40) with respect to the regulation of Ca\(^{2+}\) homeostasis.

MATERIALS AND METHODS

Preparation of Synaptosomes—The cortices of male Wistar rats (200–250 g) were dissected and homogenized in ice-cold 0.2 M sucrose (10 strokes, Teflon-glass homogenizer). The homogenate was centrifuged at 1,500 \(\times\) g for 5 min at 4 °C. The resulting supernatant was centrifuged at 15,000 \(\times\) g for 15 min at 4 °C. The resultant pellet, which yielded impure synaptosomal preparation P1, was resuspended in incubation buffer containing the following (in mM): NaCl 128, KCl 4.8, KH\(_2\)PO\(_4\) 1.2, MgSO\(_4\) 1.2, NaHCO\(_3\) 7.5, glucose 10, ascorbic acid 0.1, HEPES 15, CaCl\(_2\) 1.3, sodium EDTA 0.03. The synaptosomal preparation was then incubated with \(\beta\)A-(1–40) (1 \(\mu\)M) for 1 h at 30 °C prior to measuring 45Ca\(^{2+}\) influx or [Ca\(^{2+}\)].

Culture of Cortical Neurons—The effects of \(\beta\)A-(1–40) and IL-1\(\beta\) on the Ca\(^{2+}\) channel current in primary cultures of rat cortical neurons were investigated using the whole-cell configuration of the patch-clamp technique. Cells were obtained by enzymatic and mechanical dissociation as described previously (12). Briefly, cortices were removed from 16–18-day fetal rats and triturated following trypsin digestion. Suspended cells were plated out at a density of 0.25 \(\times\) 10\(^{6}\) cells/mm\(^2\) in circular coverslips (10-mm diameter) and incubated in a humidified atmosphere containing 5% CO\(_2\), 95% air at 37 °C. After 48 h 80 \(\mu\)M fluoro-deoxyoxuridine was included in the culture medium to prevent proliferation of non-neuronal cells. The culture medium was exchanged every 3 days, and cells were grown in culture for up to 14 days. All recordings were made from cells between days 5 and 12.

Measurement of 45Ca\(^{2+}\) Influx—20 \(\mu\)l of the P2 synaptosomal preparation \(\pm\) \(\beta\)A (1 \(\mu\)M) was suspended in 60 \(\mu\)l of oxygenated incubation buffer containing 45Ca (final concentration, 1 \(\mu\)Ci/ml; specific activity, 2.1 mCi/ml; Amersham Pharmacia Biotech) and either 4.8 mM KCl or 50 mM KCl for analysis of unstimulated and stimulated 45Ca\(^{2+}\) influx, respectively. The synaptosomes were then incubated for 5 at 37 °C. In some experiments IL-1\(\beta\) (5 ng/ml) or vehicle (Krebs-buffered saline) was included in the 4.8 mM KCl and 50 mM KCl incubation buffers. In
experiments involving the Ca$^{2+}$ channel blockers nifedipine (10 µM) and ω-conotoxin-GVIA (2 µM), the blockers were also included in the incubation buffers, and dimethyl sulfoxide (0.1%) was used as a vehicle control. Reactions were stopped by addition of 1 ml of ice-cold "stop" buffer (composition in mM: NaCl 118, K$_2$HPO$_4$ 1.2, MgSO$_4$ 1.2, NaHCO$_3$ 6, CaCl$_2$ 1.3, glucose 11, ouabain 10). Samples were then rinsed under vacuum in a filtration manifold, and filters were added to scintillation fluid for assessment of radioactivity. Data were expressed as nanomoles of 45Ca$^{2+}$/mg protein.

**Determination of [Ca$^{2+}$]i, in Synaptosomes—Fura-2/AM (Molecular Probes, The Netherlands) was stored at −20 °C in a 1 nM stock solution in dimethyl sulfoxide. The synaptosomal suspension (5 µM) was incubated with Fura-2/AM (2 µM) for 1 h at 30 °C in oxygenated Krebs buffer (composition in mM: NaCl 139, K$_2$HPO$_4$ 1.2, MgSO$_4$ 1.2, NaHCO$_3$ 16, glucose 10, CaCl$_2$ 2). The Fura-2-loaded synaptosomes were centrifuged at 5,000 × g for 1 min, and the pellet was resuspended in Krebs to a final protein concentration of 1 mg/ml. The [Ca$^{2+}$]i, measurements were recorded as described previously (13). Briefly, 2 ml aliquots of Fura-2-loaded synaptosomal suspension were placed in the cuvette holder of a Cairn spectrophotometer (Cairn, Faversham, UK). Experiments were performed at 37 °C. Using the ratio of fluorescence at 340 and 380 nm, with a constant emission at 510 nm, the change in [Ca$^{2+}$]i, was determined as described previously (14). The maximum fluorescence ratio (R$_{\text{max}}$) was achieved by lysing the synaptosomes with 5 µl of 10% Triton X-100, and the minimum fluorescence ratio (R$_{\text{min}}$) was achieved by addition of 50 µl of 0.5 M EGTA with 2 M Tris/HCl, pH 8.4. Correction for autofluorescence of the synaptosomal preparation was routinely made prior to making final [Ca$^{2+}$]i, determinations. Following correction of resting [Ca$^{2+}$]i, the synaptosomal suspension was depolarized by addition of KCl (50 mM) in order to evaluate the KCl-induced rise in [Ca$^{2+}$]i.

**Electrophysiological Recording—**For recording Ca$^{2+}$-currents, patch pipettes were filled with solution containing (in mM) Cs-HEPES 100, EGTA 30, CaCl$_2$ 3, MgCl$_2$ 2.5, K$_2$ATP 3.25; osmolarity was 320 mosM with sucrose; pH was adjusted to 7.2 with CsOH. Cells were bathed in a solution that consisted of (in mM) tetraethylammonium acetate 70, N-methyl-d-glucamine 70, KOH 3, magnesium acetate 0.6, glucose 4, sodium acetate 10, HEPES 10, and tetrodotoxin 0.0005; pH was 7.4 with acetic acid; osmolarity was 320 mosM with sucrose. To record Ca$^{2+}$-current-voltage relationships, cells were held at a potential of either −90 or −50 mV and depolarized to potentials ranging from −60 to +60 mV. The steps were repeated every 10 s. For all electrophysiological recordings, five leak subtraction steps were made prior to depolarization to allow off-line removal of linear leak and residual capacitive currents. Resting current was also measured to ensure that changes in leak did not affect the results. Current-voltage relationships for the Ca$^{2+}$-channels in cortical neurons were recorded following 24-h preincubation with either 1 µM Aβ-(1–40) or 1 µM Aβ-(40–1) as controls. No Aβ was present in the recording medium when Ca$^{2+}$-current channel currents were measured.

**Results—**The two batches of Aβ-(1–40) were obtained from Sigma and Bachem (Saffron Walden, UK). Aβ was dissolved in deoxygenated, deionized water at 100 µM, aliquoted to 10 µM, and stored at −20 °C until needed. Stock peptide was stored as powder at −20 °C. Aβ-(40–1) was prepared in exactly the same way. Ca$^{2+}$-channel antagonists ω-conotoxin GVIA (Peninsula Laboratories, St. Helens, UK), nifedipine (Toceis Neuramin, Bristol, UK), and ω-agatoxin IVA (Sigma) were made up as stock solutions in water before the appropriate dilution in recording medium. All culture reagents were obtained from Life Technologies, Inc. except for chick embryo extract, which was obtained from Imperial Laboratories (UK).

**RESULTS**

Aβ-(1–40) Increases 45Ca$^{2+}$ Influx and Elevates [Ca$^{2+}$]i, in Cortical Synaptosomes—Fig. 1 demonstrates that the 45Ca$^{2+}$ influx evoked following depolarization with 50 mM KCl is significantly enhanced in synaptosomes treated with Aβ-(1–40). In control synaptosomes, 45Ca$^{2+}$ influx is significantly increased from 1.08 ± 0.141 nmol/mg of protein (mean ± S.E.) to 1.55 ± 0.23 nmol/mg of protein following K$^+$ depolarization (p < 0.05, paired t test, n = 30). In the synaptosomes pretreated with Aβ, 45Ca$^{2+}$ influx was increased from 1.44 ± 0.132 to 2.48 ± 0.154 nmol/mg of protein following K$^+$ depolarization (p < 0.01, paired t test, n = 30).

The results obtained from the Fura-2/AM experiment are shown in Fig. 2. In control synaptosomes [Ca$^{2+}$]i, is increased from 245 ± 56 nm (mean ± S.E.) to 499 ± 67 nm following K$^+$ depolarization (p < 0.01, paired t test, n = 8). In synaptosomes pretreated with Aβ-(1–40) the K$^+$-induced rise in [Ca$^{2+}$]i, is significantly greater than that observed in control synaptosomes (p < 0.01, paired t test, n = 8); thus, in Aβ-treated synaptosomes mean basal [Ca$^{2+}$]i, is significantly increased from 332 ± 53 to 730 ± 120 nm following K$^+$ depolarization (p < 0.01, paired t test, n = 8).

Identification of the VDCC Subtype Involved in the K$^+$-induced Rise in 45Ca$^{2+}$ Influx—To identify the nature of the VDCC subtype involved in the Aβ-induced rise in K$^+$-stimulated 45Ca$^{2+}$ influx, the cortical synaptosomes were treated with the L-type VDCC inhibitor nifedipine (10 µM) and the N-type VDCC inhibitor ω-conotoxin GVIA (2 µM). Fig. 3 illustrates the effect of these VDCC inhibitors on the K$^+$-stimulated rise in 45Ca$^{2+}$ influx in control and Aβ-(1–40)-treated synaptosomes. In control synaptosomes the K$^+$-stimulated rise in 45Ca$^{2+}$ influx is significantly reduced from 0.386 ± 0.065 to 0.252 ± 0.056 nmol/mg of protein (p < 0.05, paired t test, n = 10) and 0.164 ± 0.08 nmol/mg of protein (p < 0.05, paired t test, n = 10) following treatment with nifedipine (10 µM) and ω-conotoxin GVIA (2 µM), respectively.

Aβ caused a 2-fold increase in the K$^+$-stimulated rise in 45Ca$^{2+}$ influx compared with the control synaptosomal preparation (p < 0.01, paired t test, n = 10). Furthermore, in Aβ-treated synaptosomes the K$^+$-stimulated rise in 45Ca$^{2+}$ influx is also significantly reduced from 1.22 ± 0.21 to 0.186 ± 0.099 nmol/mg of protein (p < 0.01, paired t test, n = 10) and 0.176 ± 0.06 nmol/mg of protein (p < 0.01, paired t test, n = 10) following treatment with nifedipine (10 µM) and ω-conotoxin GVIA (2 µM), respectively.

**Effect of IL-1β on 45Ca$^{2+}$ Influx—**To assess the effect of IL-1β on 45Ca$^{2+}$ influx the control and Aβ-(1–40)-treated synaptosomes were exposed to IL-1β (5 ng/ml) during the 5-s K$^+$ depolarization phase. Fig. 4 illustrates the effect of IL-1β on K$^+$-stimulated 45Ca$^{2+}$ influx. In control synaptosomes K$^+$-
Effect of IL-1β on K⁺-stimulated ⁴⁵Ca²⁺ influx in cortical synaptosomes. In control synaptosomes ⁴⁵Ca²⁺ influx was stimulated by a 5-s exposure to KCl (50 mM; black bar). Exposure of the synaptosomes to IL-1β (5 ng/ml; gray bar) during the depolarization phase resulted in a significant reduction in K⁺-stimulated ⁴⁵Ca²⁺ (*, p < 0.05). In synaptosomes pretreated with β-amyloid (1 μM, 1 h at 30°C) the ⁴⁵Ca²⁺ influx resulting from a 5-s exposure to KCl (50 mM; black bar) was significantly greater than that observed in control synaptosomes (p < 0.05). In addition, exposure of Aβ-(1–40)-treated synaptosomes to IL-1β (5 ng/ml; gray bar) during the K⁺ depolarization phase caused a significant reduction in K⁺-stimulated ⁴⁵Ca²⁺ influx (p < 0.01). Results are expressed as the mean ± S.E. of 10 observations.

Effect of Aβ-(1–40) on Cortical Neuron Ca²⁺ Channel Currents—Exposure of cortical neurons to 1 μM Aβ-(1–40) for 24 h resulted in an increase in Ca²⁺ channel current when compared with controls. Maximal inward Ca²⁺ channel current was increased by 49% when step-depolarizing cells to the +10 mV potential from a holding potential of −90 mV (Fig. 5, control, −94.5 ± 10.0 pA/pF, n = 39; Aβ-(1–40), −141.1 ± 11.6 pA/pF, n = 47, p < 0.01). Using a holding potential of −50 mV, peak Ca²⁺ channel current was increased by 64% at the +10 mV potential (control, −40.7 ± 8.1 pA/pF, n = 37; Aβ-(1–40), −66.7 ± 7.4 pA/pF, n = 31, p < 0.01). At this potential only Ca²⁺ channel currents that are relatively resistant to inactivation by depolarization would be expected to be activated. Thus the Ca²⁺ current that is increased by Aβ in cortical neurons appears, in some degree, to be resistant to inactivation. Whole cell conductance and leak current values were also measured during recordings and were found to be unchanged.

To identify the Ca²⁺ channel subtype augmented by preincubation of cortical neurons with Aβ, subtype-selective Ca²⁺ channel blockers were included in the recording medium during measurement of Ca²⁺ channel activity. Fig. 5 shows Ca²⁺ channel current density evoked at the +10 mV potential when recordings were made in the presence of various Ca²⁺ channel antagonists.

The L-Type Ca²⁺ channel blocker nimodipine (2 μM) slightly reduced Ca²⁺ channel current density when compared with untreated cells but had little impact on the increase in Ca²⁺ channel current density induced by Aβ (control, −84.9 ± 11.2 pA/pF, n = 13; Aβ-(1–40), −128.6 ± 14.4 pA/pF, n = 16, p < 0.01), suggesting that L-type channels were not augmented by preincubation with Aβ.

ω-Conotoxin GVIA (1 μM) attenuated currents in untreated cells by approximately 60%, indicating the presence of a large N-Type Ca²⁺ channel component. Furthermore, Aβ appeared to interact with N-type Ca²⁺ channels as ω-conotoxin GVIA greatly reduced the increase in Ca²⁺ channel current density induced by Aβ. However, a small (31%), statistically significant increase in Ca²⁺ channel current was observed at the +10 mV potential (control, −37.0 ± 3.0 pA/pF, n = 42; Aβ-(1–40), −48.3 ± 4.8 pA/pF, n = 39, p < 0.05, t test). As ω-conotoxin GVIA was unable to completely prevent the increase in current...
The importance of the P-type component Ca²⁺ channel current in cortical neurons was shown by a 30% reduction in control Ca²⁺ channel current by 30 nM ω-agatoxin IVA. After increasing the Ca²⁺ channel current with Aβ, ω-agatoxin IVA (30 nM) blocked the current by approximately 45%. When Ca²⁺ channel currents measured in the presence of ω-agatoxin IVA in Aβ-(40–1)- and Aβ-(1–40)-treated cells were compared, it was found that Aβ no longer increased the Ca²⁺ channel current (control, −66.5 ± 7.0 pA/pF, n = 22; Aβ-(1–40), −79.5 ± 9.7 pA/pF, n = 20). This suggests that P-type channels are also modulated by Aβ.

Attenuation of ⁴⁵Ca²⁺ influx in synaptosomes by IL-1β suggested that we would be able to block the increased current in rat cortical neurons with IL-1β. Currents from Aβ-(40–1)- and Aβ-(1–40)-treated cells were measured in the presence of 5 ng/ml IL-1β. IL-1β significantly inhibited the Ca²⁺ channel current following both treatments. Furthermore, Aβ was unable to cause a significant increase in Ca²⁺ channel current density in the presence of IL-1β when cells were depolarized from a holding potential of −90 mV (control, −51.9 ± 5.7 mV, n = 12; Aβ-(1–40), −60.4 ± 4.4 mV, n = 12, Figs. 5 and 6A). There was also no effect of Aβ on Ca²⁺ channel current density when cells were depolarized from a holding potential of −50 mV (Fig. 6B). The decrease in Ca²⁺ channel current density observed in the presence of IL-1β represents a reduction of approximately 45%. These data support the previous finding that IL-1β can attenuate the effects of Aβ on ⁴⁵Ca²⁺ influx in synaptosomes.

**DISCUSSION**

The aim of this study was to examine the effects of Aβ-(1–40) and the proinflammatory cytokine IL-1β on Ca²⁺ homeostasis in cortical synaptosomes and cultured cortical neurons. The results demonstrate that Aβ-(1–40) stimulates ⁴⁵Ca²⁺ influx into the synaptosomal preparation with a parallel rise in [Ca²⁺]. The electrophysiological data demonstrate that Aβ-(1–40) also increases voltage-dependent Ca²⁺ channel activity in cultured cortical neurons. Since the stimulatory effects of Aβ-(1–40) on ⁴⁵Ca²⁺ influx into cortical synaptosomes were blocked by nifedipine and ω-conotoxin GVIA we conclude that L- and N-type VDCCs are involved in the Aβ-induced elevation in ⁴⁵Ca²⁺ influx in this preparation. In the cultured cortical neurons the Aβ-induced elevation in Ca²⁺ channel current was found to be sensitive to ω-conotoxin GVIA and ω-agatoxin IVA, indicating the involvement of N- and P-type VDCCs. The data also demonstrate that the proinflammatory mediator IL-1β attenuates ⁴⁵Ca²⁺ influx into cortical synaptosomes and reduces Ca²⁺ channel current density in cultured cortical neurons by approximately 50%, providing evidence that cortical voltage-sensitive Ca²⁺ channels are substrates for modulation by IL-1β. Furthermore, exposure to IL-1β was found to prevent the stimulatory effects of Aβ-(1–40) on Ca²⁺ influx, and this result is indicative of an interaction between Aβ-(1–40) and IL-1β with respect to the regulation of Ca²⁺ homeostasis.

Our finding that Aβ-(1–40) stimulated ⁴⁵Ca²⁺ influx and elevated [Ca²⁺], at the level of the nerve terminal means that Aβ-(1–40) is likely to have a significant impact on Ca²⁺-dependent neuronal functions such as neurotransmitter release and synaptic plasticity. In support of this hypothesis, β-amylloid peptides have been shown to potentiate Ca²⁺-dependent release of glutamate and aspartate from hippocampal slices (15), impair long term potentiation in the hippocampus (16), and alter spatial memory performance tests (17). Given that elevations in [Ca²⁺], have been shown to precede apoptotic events in several cell systems (18), the stimulation of ⁴⁵Ca²⁺ influx and enhancement of VDCC activity by Aβ-(1–40) may act as a trigger for the neuronal apoptosis associated with neurodegenerative disease. In support of this suggestion, Aβ-induced increases in Ca²⁺ influx are reported to be involved in mediating the apoptotic effects of Aβ in PC12 cells (19, 20), and L-type VDCCs have been demonstrated to mediate Aβ-induced neurotoxicity in cultured hippocampal neurons (21). In addition, mobilization of intracellular Ca²⁺ stores via formation of reactive oxygen species has been suggested to underlie the detrimental effects of Aβ-(25–35) on synaptosomal membrane lipid structure and composition (22). Although elevation in [Ca²⁺], has been demonstrated to underlie Aβ-induced neuronal apoptosis, Aβ also stimulates glia to produce growth factors that contribute to plaque development (5) and may influence neuronal viability (23). In addition, the ability of Aβ to induce expression of adhesion molecules (24) may also play a significant role in the pathogenesis of AD.

The subtype of VDCC modulated by Aβ-(1–40) in the cortical synaptosomes and cultured neurons was investigated using selective Ca²⁺ channel blockers. The L-type VDCC blocker nifedipine and the N-type VDCC inhibitor ω-conotoxin GVIA were both found to attenuate the K⁺-stimulated ⁴⁵Ca²⁺ influx in control cortical synaptosomes, indicating that L- and N-type VDCCs become activated following K⁺ depolarization. The finding that ω-conotoxin GVIA blocked K⁺-stimulated ⁴⁵Ca²⁺ influx in the synaptosomal preparation is consistent with a presynaptic role for the N-type VDCC because N-type VDCCs have been localized to nerve terminals, where they play a critical role in presynaptic events such as neurotransmitter release (25). In addition, a presynaptic role for L-type VDCCs has been described in cerebellar granule neurons (26), and our finding that nifedipine attenuated the K⁺-induced increase in ⁴⁵Ca²⁺ influx in the synaptosomal preparation is consistent with previous studies that demonstrate the presence of L-type VDCCs in cortical synaptosomes (27). The finding that nifedipine and ω-conotoxin GVIA also blocked the Aβ-mediated poten-
tiation of the K⁺-induced increase in ⁴⁵Ca²⁺ influx in cortical synaptosomes suggests that N- and L-type VDCCs are targets for presynaptic modulation by Aβ-(1–40). In contrast, in the cultured cortical neurons a 20-ms, 110-mV depolarization from a holding potential of −90 mV resulted in activation of N- and P-type VDCCs, with little contribution from L-type VDCCs, demonstrating that N- and P-type VDCCs are prevalent in cultured cortical neurons. The Aβ-(1–40)-induced increase in Ca²⁺ channel current density was significantly reduced by ω-conotoxin GVIA and the P-type VDCC blocker ω-agatoxin IVA but was unaffected by the L-type VDCC blocker nimodipine, indicating that N- and P-type VDCCs are modulated by Aβ-(1–40) in these cells. In cerebellar granule neurons Aβ has been shown to act via exclusive up-regulation of N-type channels (28) and L-type VDCCs in microglia (29); thus the nature of the VDCC regulated by Aβ may be dependent upon the cell type and subcellular fraction under investigation.

We found that IL-1β attenuated ⁴⁵Ca²⁺ influx in cortical synaptosomes in a similar manner to that previously reported in synaptosomes prepared from the hippocampus (30). IL-1β functions as a neuromodulator to reduce neurotransmitter release (30) and impair long term potentiation in the hippocampus (31) possibly as a consequence of its inhibitory effect on Ca²⁺ influx (30). The finding that IL-1β reduces Ca²⁺ channel current in cultured cortical neurons is also similar to the modulatory effects reported for IL-1β on hippocampal Ca²⁺ channel currents, where the IL-1β effects were found to proceed via activation of a pertussis toxin-sensitive G-protein (32). Modulatory effects of IL-1β on Ca²⁺ homeostasis in cortical neurons and cortical nerve terminals are likely to have a significant impact on cortical function and may underlie the modulatory effects of IL-1β on synaptic transmission in the neocortex (33).

The finding that IL-1β blocked the stimulatory effects of Aβ-(1–40) on ⁴⁵Ca²⁺ influx and Ca²⁺ channel currents is of particular interest. Elevations in neuronal expression of both Aβ (2) and IL-1β (3) are pathological features of Alzheimer’s disease, and several lines of evidence demonstrate an interaction between Aβ and IL-1β. β-Amyloid induces release of IL-1β from activated microglia (34) and monocytes (35) in a Ca²⁺-dependent manner and also promotes transcription of IL-1 mRNA (36). Aside from the association of IL-1 with amyloid plaques (37), where it is thought to contribute to gliosis, IL-1 is also believed to be influential at an earlier phase of AD pathology because this cytokine has been found to enhance βAPP mRNA expression in neurons (6) and promote processing of βAPP to generate β-amyloid fragments (7). IL-1β and Aβ therefore participate in a cycle of events contributing to the formation of the neuritic plaques and tangles associated with AD. Although those studies reveal an interaction between Aβ and IL-1β at a processing level, little information is currently available concerning a physiological interaction between Aβ and IL-1β. The results presented in this study demonstrate that IL-1β modulates the Aβ-(1–40)-induced stimulation of Ca²⁺ influx in the cortex, and such neuromodulatory properties of IL-1β may participate in governing neuronal responses to Aβ at certain stages of the neurodegenerative disease process. Thus, whereas IL-1β is considered to have a significant influence on the pathophysiology of AD by increasing βAPP expression resulting in the formation of neuritic plaques (4), the proclivity of IL-1β to attenuate Aβ-induced Ca²⁺ influxes at the level of the nerve terminal may impact on presynaptic function, such as the release of excitatory neurotransmitters, to limit cell dam-

FIG. 6. Effect of Aβ-(1–40) on Ca²⁺ channel current density in cultured rat cortical neurons in the presence of IL-1β. Effect of 1 μM Aβ-(1–40) on Ca²⁺ channel current density in cultured rat cortical neurons in the presence of 5 ng/ml IL-1β. A, mean current/voltage relationships for cells incubated for 24 h with either 1 μM Aβ-(1–40) (filled triangles, n = 12) or 1 μM of the reverse sequence (open circles, n = 12). No significant differences between the currents at each test potential could be detected (Student’s unpaired t test). Mean peak Ca²⁺ current density is plotted against the test potential evoking the current when cells were depolarized from a holding potential of −90 mV. Inset, averaged current traces, which are not corrected for capacitance, from the same cells shown in A at the +20 mV potential. B, mean current/voltage relationships when Ca²⁺ channel current was evoked by depolarizing cells from a holding potential of −50 mV (Aβ-(1–40), filled triangles, n = 17; Aβ-(40–1), open circles, n = 17). No significant differences between the currents at each test potential could be detected (Student’s unpaired t test). Inset, averaged current traces from the same cells shown in B at the +20 mV potential.
age caused by glutamate excitotoxicity, and as such this may reflect a neuroprotective role for IL-1β. Furthermore, because Aβ-induced neuronal apoptosis is dependent upon the activation of Ca²⁺-sensitive proteases (38) and Ca²⁺-sensitive tyrosine kinases (39), our observation that IL-1β occludes the stimulatory effects of Aβ on Ca²⁺ influx suggests that IL-1β may serve to restrain these intracellular cascades associated with Aβ-induced neurodegeneration.

In summary, the increase in Ca²⁺ influx mediated by Aβ-(1–40) in cortical synaptosomes and cultured cortical neurons is attenuated by the proinflammatory cytokine IL-1β. This finding indicates an interaction between Aβ and IL-1β that serves to regulate Ca²⁺ homeostasis, and this interaction may have implications in the manifestation of AD.

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Aoife MacManus, Martin Ramsden, Michael Murray, Zaineb Henderson, Hugh A. Pearson and Veronica A. Campbell

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