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Publication details:
BBA - Biochimica et Biophysica Acta
v. 1822
Chapter No. 8
pp. 1247-1257
0006-3002 (ISSN)

Publication Date:
2012

Publisher DOI:
http://dx.doi.org/10.1016/j.bbadis.2012.04.007

Role of hippocalcin in mediating Aβ toxicity

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Author contribution: Y-AL, AE, JG designed the experiments. Y-AL and MG performed the experiments; Y-AL, AE and JG analyzed and discussed the data; Y-AL and AE performed statistics; CS, GH, MK and MS provided materials and input to discussion; and Y-AL and JG wrote the paper.

Abstract (250 words)

Alzheimer's disease (AD) is the most common cause of dementia. There is no cure available and the current treatments provide only limited symptomatic relief. Histopathological hallmarks of the AD brain are the amyloid-beta (Aβ) plaques and the tau-containing neurofibrillary tangles. These Aβ and tau lesions do not occur at random, rather, the neurodegenerative process is stereotyped in that it is initiated in the entorhinal cortex and hippocampal formation. Interestingly, it is the latter brain area where the calcium-sensing enzyme hippocalcin is highly expressed. As calcium deregulation in the form of excitotoxicity is a well-established pathomechanism in AD, we aimed to address the putative role of hippocalcin in human AD brain and transgenic mouse models. We found that levels of hippocalcin are higher in human AD brains and in Aβ plaque-forming APP23 transgenic mice compared to controls. In APP23 mice there was an inverse relationship between Aβ and hippocalcin staining as those cells with the highest intracellular Aβ had very low levels of hippocalcin. To determine the role of hippocalcin in Aβ toxicity, we treated primary cultures derived from mice lacking hippocalcin with Aβ and found that they were more susceptible to Aβ toxicity than the controls. Likewise, treatment with thapsigargin and ionomycin, respectively, both of which are known to deregulate intracellular calcium levels, caused an increased toxicity in hippocampal neurons from hippocalcin knock-out mice compared to wild-type controls. Our findings suggest that hippocalcin has a neuroprotective role in AD. The data further suggest hippocalcin as a putative biomarker for AD.

Introduction

Alzheimer's disease (AD) is the most common form of dementia, affecting approximately 26 million people worldwide [1]. Histopathologically, the AD brain is characterized by the deposition of amyloid-β (Aβ) plaques and tau-containing neurofibrillary tangles in the brain [2-5]. At present, there is no cure for this debilitating disease [1]. Of all structures in the brain, the hippocampus is one of the earliest to be affected in AD, reflecting the fact that it is the formation of new memories that is impaired at an early stage of disease [6, 7]. Furthermore, the hippocampus is a brain area that has been shown to be a major target of excitotoxic damage, whereby neurons die because of an excessive stimulation of glutamate receptors [8].

Hippocalcin is a protein that is highly expressed in the hippocampus [9]. It is a member of the neuronal-specific calcium sensor (NCS) family of proteins, and as such by employing a calcium-activated myristoyl switch is translocated to the plasma membrane upon binding calcium [10]. The expression pattern of hippocalcin differs from that of the other known members of the protein family, with hippocalcin being mainly expressed in the hippocampus, while the other members are mainly found in other brain regions [11]. This suggests that hipocalcin it has a unique function that is not shared by the other family members. Hippocalcin has been shown to be important for the homeostasis of intracellular calcium levels [12]. Infusion of a truncated mutant form of hippocalcin that lacks the Ca^{2+} binding domains has been shown to prevent synaptically evoked LTD (long term depression) but had no effect on LTP (long term potentiation) [13]. To better understand the function of the calcium sensor hippocalcin, knock-out mice have been generated and shown to display memory deficits in spatial recognition tasks, further suggesting that the protein plays a role in memory formation [14].

Hippocalcin has been found to exert a neuroprotective effect *in vitro*, such as in the spinal cord motor neuron-like cell line NSC-34 or the neuroblastoma cell line Neuro-2a, by interacting with NAIP (neuronal apoptosis inhibitory protein) or by promoting calcium extrusion [15, 16]. There is also histopathological evidence for a role of hippocalcin in neurodegeneration, because of the protein's localization to Lewy bodies, insoluble aggregates present in Parkinson's disease brains, which suggests that

hippocalcin may play a role in the pathogenesis of this condition [17]. In Huntington's disease, on the other hand, hippocalcin levels were found to be decreased [18]. Together, this supports the notion that hippocalcin is deregulated under neurodegenerative conditions.

The present study aimed to determine the role of hippocalcin levels in Alzheimer's disease brain and in an AD mouse model with amyloid plaque formation. We further addressed its role in Aβ toxicity using primary neuronal cultures that have been established from hippocalcin knock-out and wild-type mice.

Material and methods

Ethics statement

Human brain tissue was obtained from the Australian Brain Bank Network's Sydney Brain Bank, with approval from the Human Ethics Review Committee of the University of Sydney. Written informed consent was obtained from donors or donors next of kin for brain donation. The animal experiments were approved by the Animal Ethics Committee (AEC) of the University of Sydney (approval number K00/1-2009/3/4914).

Immunofluorescence staining

The left hemispheres of APP23 transgenic mice and age-matched wild-type littermates controls after PBS perfusion, were post-fixed in 4% paraformaldehyde overnight at 4°C and the paraformaldehyde exchanged for 70% ethanol the next day. The brains were then processed in a Shandon Excelsior processor (Thermo), followed by embedding in paraffin. 3 µm sections were rehydrated step-wise before antigen retrieval was performed in a microwave system (Milestone) in pre-warmed Tris/EDTA at pH 9.0 for 7 minutes at 120°C and allowed to cool on the bench-top for 15 minutes. Sections were stained with 6E10 (1:500, Covance), hippocalcin (1:100) [19] and counterstained with DAPI (Invitrogen) to visualize the nuclei. Alexa-Fluor secondary antibodies (Alexa-Fluor 488

and Alexa-Fluor 555, respectively, Invitrogen) were used for detection. Fluorescent pictures of the stained sections were taken on a Zeiss LSM 710 confocal microscope.

Transgenic mice and protein extraction

Six and 18 month-old transgenic APP23 mice and age-matched wild-type littermate controls [20] were transcardially perfused with 1 x PBS, followed by separation of the hemispheres. The left hemisphere was post-fixed in 4% paraformaldehyde overnight at 4°C, while the right hemisphere and the hippocampus were sub-dissected and snap frozen in liquid nitrogen for Western blotting. Protein extraction was performed on mouse hippocampal tissue using RIPA buffer (50 mM Tris, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate 1% Triton-X with protease inhibitors). Tissue was sonicated on ice to obtain a homogeneous suspension. The lysates were spun down at 17,000 x g for 20 minutes at 4°C. The supernatant was retrieved and protein levels were determined using the Bradford assay. Samples were kept at -80°C until further use.

Human tissue and protein extraction

Frozen human hippocampal brain tissue from the inferior temporal was homogenized in Tris buffer solution (TBS) (2.5 mL of 20 mM Tris-CI, 5 mM EDTA, 0.02% sodium azide) containing protease inhibitors (Complete Mini, Roche Diagnostics). Homogenates were centrifuged at 17,000 x g at 4°C for 2 hours and the supernatant was retrieved to obtain the Tris buffer- soluble fraction. The pellet was re-suspended in TBS containing 2% SDS (2.5 mL of 20 mM Tris-CI, 5 mM EDTA, 0.02% sodium azide, 2% SDS) and protease inhibitors (Complete Mini, Roche Diagnostics) and was centrifuged again at 17,000 x g at 25°C for 30 minutes to obtain the SDS-soluble fraction, containing membrane-bound, intracellular proteins. The supernatant was retrieved and protein levels were determined using the Bradford assay and kept at -80°C until needed.

Western blotting

40 µg of protein was separated on 10% Tris-Tricine SDS gels as described previously to visualize Aβ [21]. Proteins were transferred onto nitrocellulose membranes (Hybond, GE Healthcare). To enable an accurate comparison between the different proteins of interest, blots were cut at positions 55, 38 and 17 kDa using the protein molecular weight ladder (Fermentas, Catalogue number: SM-1811) as reference, and blocked, in parallel, with 5% skim milk in 1 x TBST (Tris-Buffered Saline with 1% Tween 20) for an hour at room temperature. Primary antibodies were diluted in 5% milk/TBST and incubated overnight at 4°C, using the following antibodies: 6E10 (directed against 1-16 amino acid residues of Aβ, 1:1000, Covance), GAPDH (1:5000, Millipore), NeuN (1:1000, Millipore) and Hippocalcin (1:1000) [19]. Blots were washed 3 times with 1 x TBST (5 minutes wash each), and incubated with the respective alkaline phosphatase-tagged secondary antibodies for 1 hour at room temperature. The blots were again washed 3 times with 1x TBST (5 minute washes each), after which they were developed with Immobilon Chemiluminescent Alkaline Phosphatase substrate (Millipore), and detected using the VersaDoc Model 4000 CCD camera system (BioRad). Densitometric analyses were performed using the ImageJ software (NIH).

Primary cultures

Primary cultures were prepared from embryonic day 16 (E16) hippocalcin-knockout and wild-type mice as described previously [22]. In brief, hippocampi and cortices were dissected and trypsinized in Hank's balanced salt solution (HBSS) with 0.0008% DNase (w/v). The preparation was gently shaken and centrifuged at 720 x g for 3 minutes at room temperature to allow the brain pieces to settle at the bottom of the tube. The supernatant was aspirated and the remaining brain tissue was triturated 30 times in plating media (10% FBS in DMEM). Cell numbers were determined using a Neubauer haemocytometer (Marienfeld, Germany). 50,000 cells were plated per well. Cultures were placed in a 5% CO_2 incubator at 37°C for 2 h to allow the cortical neurons to adhere to the poly-lysine pre-coated 48-well plates, after which the plating medium was removed

and replaced with Neurobasal medium supplemented with 1% (v/v) B27 supplements (Gibco), 0.01% (v/v) 200mM L-glutamine (Gibco) to minimize growth of microglia and astrocytes. Cultures were grown for 10 days in vitro (DIV) before treatment.

Treatment and cell viability of primary cultures

After 10 days in vitro (DIV), primary hippocampal and cortical neurons were treated with different concentrations of Thapsigargin (Sigma), ionomycin (Sigma), Aβ42 (Bachem) or DMSO for 4 days. In addition, primary cultures were treated with Aβ42 to assess the role of hippocalcin during Aβ toxicity. Cell viability was assayed using MTT as described previously [23]. Background absorbance was subtracted from raw MTT absorbance readings and resulting values were normalized against DMSO vehicle treatments.

Results and Discussion

Levels of hippocalcin are inversely correlated with Aβ in APP23 mouse brain

Hippocalcin is a neuronal protein that is highly expressed in the hippocampus [19]. Since this brain area is one of the earliest brain structures where the degenerative process is initiated in AD, we investigated the expression pattern of hippocalcin in Aβ-plaque-forming APP23 mice, an established AD mouse model [24]. The APP23 strain expresses human APP together with an AD-linked KM670/671NL double mutation (Swedish mutation) [24]. In these mice, Aβ levels increase with age, and by 6 months of age, Aβ plaque formation is initiated. To investigate the hippocampal expression of hippocalcin, we used 18 month-old transgenic mice and wild-type littermate controls, as at 6 months of age, there was no intracellular Aβ detectable (data not shown). Sagittal sections of 18 month-old mice were co-stained for Aβ (with antibody 6E10) and hippocalcin [19], and counterstained with DAPI to visualize the nucleus. Confocal microscopy revealed that wild-type CA1 pyramidal neurons uniformly showed a strong reactivity for hippocalcin, while in the APP23 hippocampus, there were two types of cells revealing an inverse relationship between hippocalcin and Aβ: we found CA1 neurons that showed a strong

staining for hippocalcin (grey arrowhead), that did not stain for Aβ, and neurons that showed a strong staining for Aβ that were hippocalcin-negative (Figure 1). Intracellular Aβ has previously been correlated with neuronal loss and has been found to be an excellent predictor for synaptic dysfunction and neuronal loss in AD [25-27]. Previously, increased levels of intracellular calcium have been found to promote the formation of oligomeric forms of Aβ [28, 29]. Interestingly, increased calcium levels also promote the aggregation of α-synuclein that forms aggregates in Parkinson's disease [30]. As it is established that hippocalcin protects neurons by enhancing calcium extrusion [16] the inverse relationship in hippocampal neurons expressing hippocalcin and Aβ suggests that Aβ may cause reductions in the calcium sensor hippocalcin preventing it from conferring neuroprotection. It is known that there are less plaques in the hippocampal formation compared to the cortex in APP23 mice, despite comparable expression levels in the two brain areas [31]. Whether this is because hippocalcin confers initial protection from plaque formation in the hippocampus, which is not available to the cortex, where hippocalcin is not expressed, remains to be determined.

Hippocalcin protein levels in APP23 mice is higher than wild-type control mice and increases with age

To determine whether the presence of Aβ is responsible for changes in hippocalcin levels as observed by immunohistochemistry, we performed Western blotting on protein extracts obtained from brains of 6 and 18 months-old APP23 transgenic and wild-type littermate controls. We determined NeuN levels as a loading control, as this neuronal nuclear protein has been found to correlate well with the number of viable neurons [32, 33]. We found that levels of NeuN (reflecting neuronal numbers) were significantly decreased in APP23 transgenic compared to wild-type hippocampi at both 6 and 18 months of age ($p<0.05$ each), reflecting the fact that the transgenic mice undergo neurodegeneration as determined previously (Figure 2A)[31, 34]. APP levels were not significantly different for both age groups in the APP23 hippocampus (Figure 2B), suggesting that their levels are not affected by aging. Aβ levels however were significantly higher in 18 months-old APP23 transgenic mice compared to 6 month-olds

(Figure 2C), as reported previously [24]. Since there was only a change in Aβ, but not APP levels, any putative change in hippocalcin levels can therefore be attributed to levels of Aβ rather than APP in these mice. We found that hippocalcin levels were increased in 18- compared to 6 months-old APP23 transgenic mice ($p<0.05$) (Figure 2D), indicating that hippocalcin levels are increased, as is Aβ (Figure 2C). In contrast, there were no significant differences in hippocalcin levels at 6 compared to 18 months in wild-type controls, further indicating that it is the AD disease state that alters hippocalcin levels (Figure 2D). Together with the immunohistochemical data, this indicates an overall increase of hippocalcin in APP mutant mice. At a cellular level there are those hippocampal neurons that massively accumulate intracellular Aβ and have very low hippocalcin levels, whereas others have higher hippocalcin levels in the absence of detectable Aβ. This may suggest that neurons upregulate hippocalcin to counteract the toxicity of Aβ as addressed below.

Hippocalcin levels vary with Aβ levels in human hippocampus brain samples

To validate the findings for hippocalcin observed in the transgenic AD mouse model in human tissue, we performed Western blotting on protein extracts obtained from the human AD hippocampus and age- and gender-matched control brain samples. NeuN was again used as a loading control as it correlates well with the number of viable neurons [32, 33]. Western blotting showed that levels of Aβ monomers (and trimers) were significantly higher in AD brains than controls (both $p<0.05$) (Figure 3A,B), while APP levels did not differ ($p=0.08$) (Figure 3C). Together, this confirmed that levels of Aβ monomers and trimers are increased in the absence of any significant changes to APP levels, as previously observed [35, 36]. NeuN levels were significantly lower in AD brains compared to controls, reflecting the neuronal loss that characterizes AD ($p<0.05$) (Figure 1D)[37]. Interestingly, after normalizing to NeuN, hippocalcin levels in AD brains were significantly increased when compared to control samples, suggesting that hippocalcin levels are upregulated in the remaining viable neurons, possibly as a protective mechanism against Aβ toxicity ($p<0.05$) (Figure 3E), since NeuN levels were previously found to be a good indicator of neuronal numbers [32] and as hippocalcin is a

neuronal protein [16, 38]. Together, our findings indicate that the surviving neurons in AD brains have higher levels of hippocalcin than neurons in healthy control brains. This suggests that hippocalcin may contribute to neuronal survival.

Hippocalcin knock-out neurons are more sensitive to calcium deregulation than wild-type neurons

To better understand the putative neuroprotective role of hippocalcin in calcium deregulation, primary cortical and hippocampal neuronal cultures were obtained from embryonic day 16 (E16) hippocalcin-knockout ([14] and wild-type mice. After 10 days *in vitro* (DIV), the neurons were treated with varying concentrations of thapsigargin (Figure 4) and ionomycin (Figure 5), both of which are known to raise intracellular calcium levels although via distinct mechanisms [39, 40].

Thapsigargin has been shown to raise the intracellular calcium concentration by preventing cells from directing calcium into the endoplasmic reticulum, thereby depleting intracellular calcium stores [41], while ionomycin affects extracellular calcium transport into cells by increasing the calcium permeability of the plasma membrane [42]. We compared hippocampal and cortical cultures from wild-type and hippocalcin knockout mice and treated the cultures for 2 days with the two compounds, followed by an MTT assay to assess cell viability.

Treatment with 0.1µM of thapsigargin revealed that wild-type hippocampal neurons were the most resistant, with a survival rate of about 75% (Figure 4A). Wild-type cortical neurons had a survival rate of less than 25%. Hippocalcin knock-out hippocampal and cortical neurons had a survival rate of 33% and 21%, respectively (Figure 4B-D). At 1.0µM thapsigargin, cell viability of wild-type hippocampal neurons was about 50% (Figure 4A), while for the knockout hippocampal neurons, they were 30% (Figure 4B-D). We did not find significant differences in cell viability between wild-type and hippocalcin knock-out cortical cells (around 20% to 30%) (Figure 4C,D), which is not surprising since hippocalcin is only expressed at very low levels in the cortex [11]. Together, our findings reveal that hippocalcin protects hippocampal neurons from the toxic effects exerted by thapsigargin.

To further assess the neuroprotective effects of hippocalcin, primary hippocampal and cortical cultures from hippocalcin knockout and wild-type mice were treated with different concentrations of ionomycin. Treatment with 0.1 µM ionomycin was not toxic to any treatment groups (Figure 5). However, at 1 µM, ionomycin was toxic only to hippocalcin-knockout hippocampal neurons ($p<0.001$) (Figure 5B), while wild-type cortical neurons, as well as hippocalcin knock-out hippocampal and cortical neurons were spared (Figure 5A,C,D). Together, this supports the notion that calcium homeostasis is critical in determining neuronal survival. Our data indicate that hippocalcin may play an important role in the maintenance of calcium homeostasis.

Hippocalcin knock-out neurons are more sensitive to Aβ toxicity

Since hippocalcin levels are consistently up-regulated in human AD and in AD transgenic APP23 mice, the putative neuroprotective role of hippocalcin in Aβ toxicity was further investigated by determining cell viability of primary hippocampal and cortical cultures from hippocalcin knockout and wild-type mice, after treatment them with varying concentrations of Aβ (Figure 6).

Treatment with Aβ showed a dose-dependent toxicity and cell viability that was decreased for all cell types by treating the cultures with Aβ concentrations between 0.5 and 5µM ($p<0.05$ to $p<0.001$) (Figure 6A,C,D), except for the hippocalcin knock-out hippocampal neurons, for which at 0.5 µM of Aβ, the cells revealed already the lowest viability of all cultures (Figure 6B). At 0.5µM Aβ, the survival rate of all cell cultures was around 65% (Figure 6A,B,D), except for hippocalcin-knockout hippocampal neurons, where it was below 55% (Figure 6B). This indicates that hippocalcin-knockout hippocampal neurons are more susceptible to Aβ toxicity. However at 5µM Aβ, the survival rates of all cell types were around 45%, obliterating any neuroprotective effects of hippocalcin.

Aβ is known to exert its toxicity via a myriad of mechanisms [3]. To determine whether the toxicity observed after exposure to Aβ is specific to Aβ rather than being a general consequence of cell death, wild-type and hippocalcin-knockout neurons were also exposed to varying concentrations of hydrogen peroxide as a source of oxidative stress,

as well as staurosporine, a non-selective protein kinase inhibitor that exerts toxicity by interfering with many toxicity pathways [43]. Importantly, for both treatments, the viability of both hippocalcin knock-out hippocampal and cortical cells did not differ from their wild-type counterparts (Figure 7), indicating that the susceptibility of hippocalcin knock-out hippocampal neurons to Aβ toxicity is specific. Together, the findings suggest that hippocalcin has a critical function in early Aβ-induced toxicity in AD.

Conclusions

Alzheimer's disease (AD) is characterized by widespread excitotoxicity, especially in the hippocampus, one of the earliest brain structures known to degenerate in AD [44]. Hippocalcin is a neuron-specific calcium sensor protein, which is highly expressed in the hippocampus and has previously been suggested to be neuroprotective [16, 38]. In this study, we found that hippocalcin levels are increased in the hippocampus of human AD and in a robust AD transgenic mouse model, indicating elevated levels of hippocalcin has a protective effect in neuronal survival. We found that hippocalcin levels were affected by intracellular Aβ levels. Our findings suggest that neurons that are capable of up-regulating hippocalcin may receive some protection, as indicated by our Western blot finding.

To investigate the neuroprotective role of hippocalcin, we had treated embryonic neuronal primary cultures In this experimental paradigm, hippocalcin-knock out hippocampal cultures showed significantly more toxicity in the presence of thapsigargin and ionomycin, indicating that hippocalcin plays an important neuroprotective role in calcium-related neuronal injury in the hippocampus. This has significant implications for other calcium-related disorders such as stroke and epilepsy [45]. Employing the same experimental system, we could also show that hippocalcin confers neuroprotection against Aβ toxicity. Collectively, our study indicates for the first time, that hippocalcin levels are deregulated by Aβ. They present hippocalcin as a biomarker and possible therapeutic target for AD.

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Figure legends

Figure 1

Confocal fluorescence analysis of 18 months-old Aβ-plaque-forming APP23 and wild-type littermate controls (WT). Paraffin-embedded sections were stained with antibodies directed against Aβ (6E10; green), hippocalcin (Hc; red) and DAPI (nuclear). Neurons that have high intracellular accumulation of Aβ have very low hippocalcin staining (white arrowhead), while neurons that have high hippocalcin levels have no detectable intracellular Aβ staining (grey arrowhead). Scale bar, 50 µm.

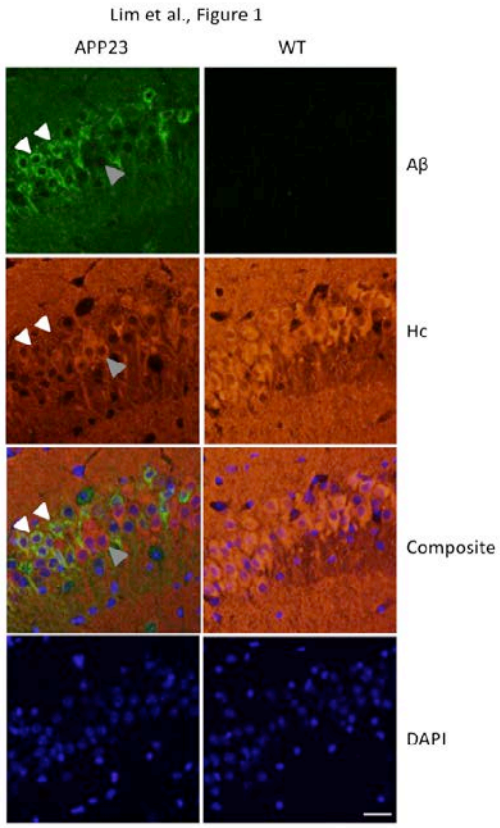


Figure 2

Western blotting of 6 and 18 months-old APP23 transgenic mice (A). There is significantly lower neuronal numbers as measured by NeuN levels between wild-type and transgenic mice at both ages (B), indicating neuronal degeneration. APP levels remain

unchanged between 6 and 18 month-old APP transgenics (C), while Aβ levels are significantly higher in 18 month-old when compared to 6 month-old transgenic mice (D). Hippocalcin levels are significantly increased from 6 to 18 months of age in the transgenic animals (E). (*, p<0.05; **,p<0.01)

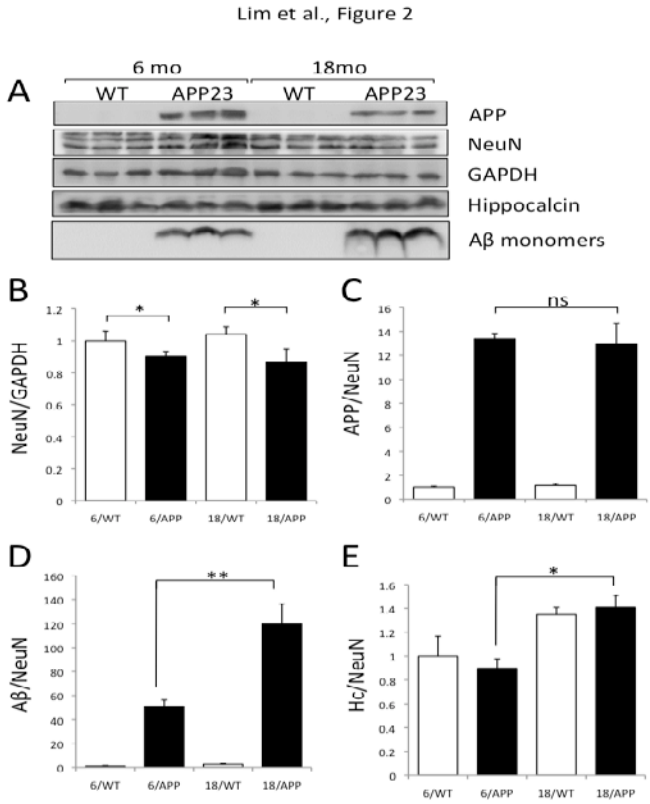


Figure 3

Western blot analysis of human AD and control (CTL) samples (A). Neuronal numbers are significantly decreased in AD samples when compared to CTL samples (B). APP levels are comparable between AD and CTL brains (C), while there is significantly higher levels of Aβ monomers and trimers in the AD brains when compared to the CTL samples (D,E). Hippocalcin levels are significantly higher in the AD brain compared to CTL samples (F). (*, p<0.05).

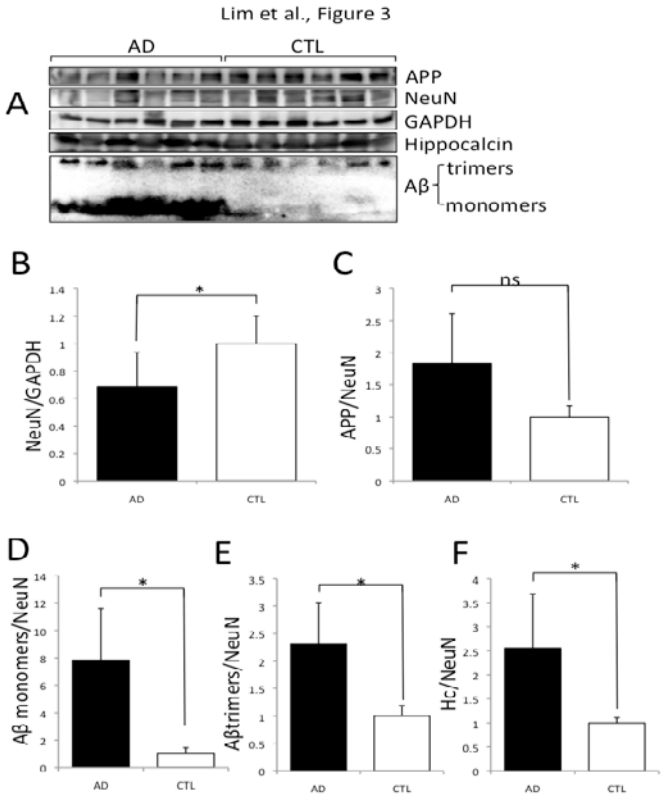


Figure 4

MTT assay to determine cell viability after thapsigargin treatment of wild-type and hippocalcin knock-out cortical and hippocampal cultures. Wild-type hippocampal cultures were the most resistant to thapsigargin toxicity and treatment with 0.1 µM and 1.0 µM thapsigargin resulted in cell viabilities of 75% and 50% respectively (A). At the same concentrations, hippocalcin knock-out hippocampal cultures had a survival rate of 33% and 30%. (B) Wild-type and hippocalcin knock-out cortical cultures were not significantly different, with cell survival rates all at around 20% (C,D). (**,$p<0.01$; ***, $p<0.001$, #, $p<0.05$)

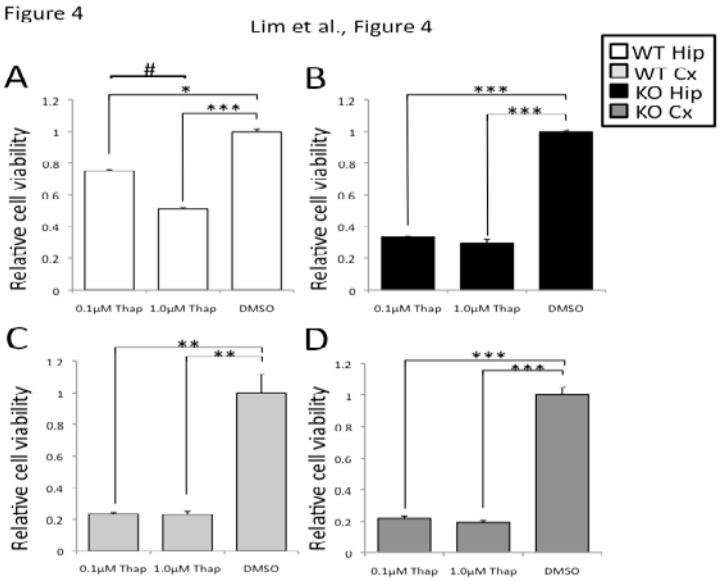


Figure 5

MTT assay for cell viability after ionomycin treatment of wild-type (A,C) and hippocalcin knock-out (B,D) cortical (C,D) and hippocampal cultures (A,B). Ionomycin treatment was not toxic to all neuronal preparations except for hippocalcin knock-out hippocampal neurons (cell survival ~70%, 5B). (***, $p<0.001$).

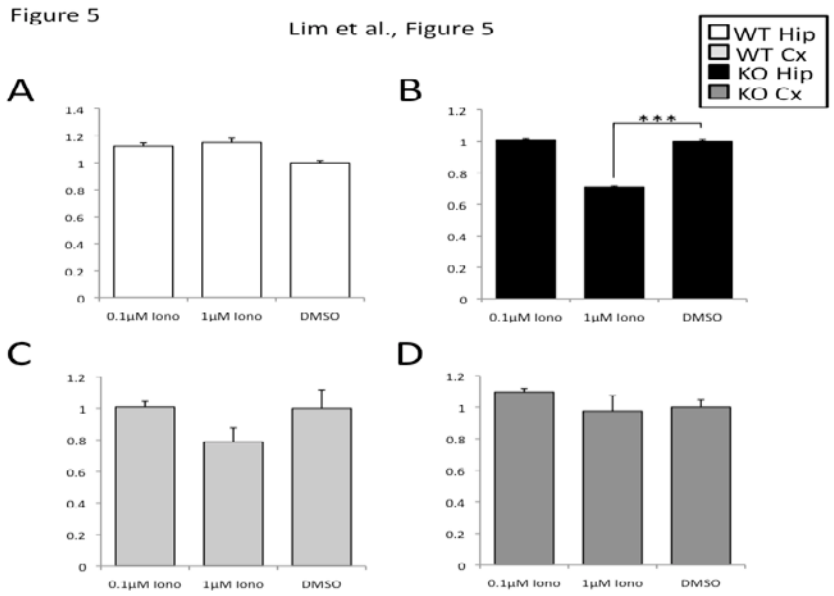


Figure 6

MTT assay for cell viability after Aβ42 treatment of wild-type (A,C) and hippocalcin knock-out (B,D) cortical (C,D) and hippocampal cultures (A,B). Aβ42 treatment induced significant cell toxicity in all neuronal cultures. Treatment with Aβ42 at 0.5 µM led to a survival rate of 65% in wild-type hippocampal neurons (A), 54% in hippocalcin knock-out neurons (B), and 63% in wild-type and hippocalcin knock-out cortical neurons (C,D). At 5 µM, cell viabilities of all neuronal preparations were below 50%. (**,$p<0.01$; ***, $p<0.001$; #, $p<0.05$; ###, $p<0.001$).

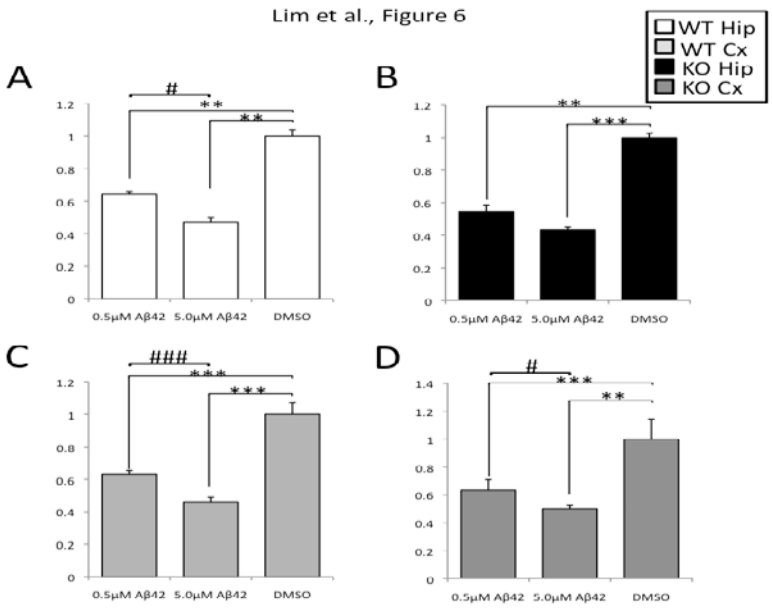
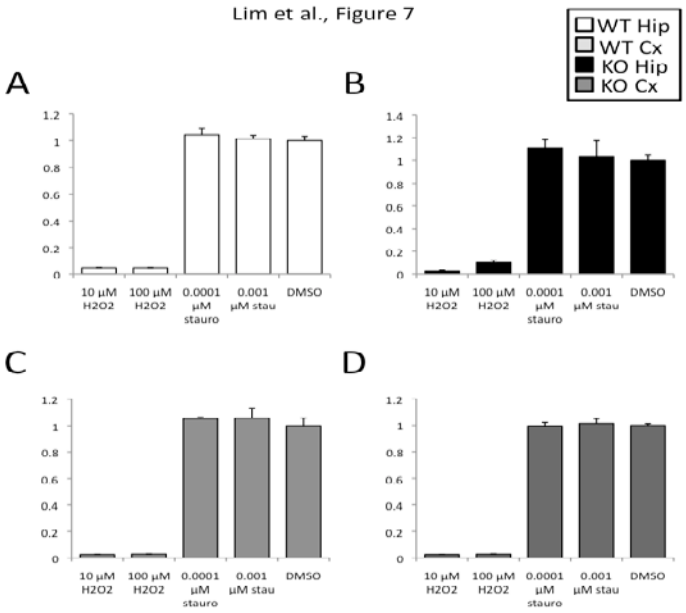


Figure 7

MTT assay for cell viability after hydrogen peroxide (H_2O_2) and staurosporine (stau) treatments of wild-type (A,C) and hippocalcin knock-out (B,D) cortical (C,D) and hippocampal cultures (A,B).. Cell viabilities of hippocalcin knock-out neurons were not significantly different from the wild-type neuronal preparations after exposure to H_2O_2 and staurosporine, indicating that hippocalcin knock-out neurons are not selectively sensitive to toxic effects exerted by H_2O_2 and staurosporine.


Lim et al., Figure 7
WT Hip
WT Cx
KO Hip
KO Cx
A
B
C
D
10 µM H2O2
100 µM H2O2
0.0001 µM stauro
0.001 µM stau
DMSO