View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Biochimica et Biophysica Acta 1812 (2011) 1584–1590

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Calcium channel blocking as a therapeutic strategy for Alzheimer's disease: The case for isradipine

Thimmappa S. Anekonda *, Joseph F. Quinn

Department of Neurology, Oregon Health & Science University, Portland, OR, USA
Portland Veteran Administration Medical Center, Oregon Health & Science University, Portland, OR, USA

ARTICLE INFO

Article history:
Received 4 May 2011
Received in revised form 12 August 2011
Accepted 30 August 2011
Available online 8 September 2011

Keywords:
Autophagy
Beta amyloid
Bioavailability
Tau

ABSTRACT

Alzheimer's disease is the most devastating neurodegenerative disorder in the elderly, yet treatment options are severely limited. The drug development effort to modify Alzheimer's disease pathology by intervention at beta amyloid production sites has been largely ineffective or inconclusive. The greatest challenge has been to identify and define downstream mechanisms reliably predictive of clinical symptoms. Beta amyloid accumulation leads to dysregulation of intracellular calcium by plasma membrane L-type calcium channels located on neuronal somatodendrites and axons in the hippocampus and cortex. Paradoxically, L-type calcium channel subtype $Ca_v1.2$ also promotes synaptic plasticity and spatial memory. Increased intracellular calcium modulates amyloid precursor protein processing and affects multiple downstream pathways including increased hyperphosphorylated tau and suppression of autophagy. Isradipine is a Federal Drug Administration-approved dihydropyridine calcium channel blocker that binds selectively to $Ca_v1.2$ in the hippocampus. Our studies have shown that isradipine *in vitro* attenuates beta amyloid oligomer toxicity by suppressing calcium influx into cytoplasm and by suppressing $Ca_v1.2$ expression. We have previously shown that administration of isradipine to triple transgenic animal model for Alzheimer's disease was well-tolerated. Our results further suggest that isradipine became bioavailable, lowered tau burden, and improved autophagy function in the brain. A better understanding of brain pharmacokinetics of calcium channel blockers will be critical for designing new experiments with appropriate drug doses in any future clinical trials for Alzheimer's disease. This review highlights the importance of $Ca_v1.2$ channel overexpression, the accumulation of hyperphosphorylated tau and suppression of autophagy in Alzheimer's disease and modulation of this pathway by isradipine.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Beta amyloid (Aβ plaque in Alzheimer's disease (AD)) accumulates long before patients become symptomatic; therefore, many therapeutic efforts have now moved to very early intervention. Another strategy to tackle the discordance between Aβ production and AD symptoms is to target mechanisms of neurodegeneration which are "downstream" of Aβ production. The greatest challenge has been to identify critical pathways that directly impact clinical symptoms and then effectively modulate these pathways by pharmacological agents. We and others have observed that a specific set of downstream pathways including dysregulation of intracellular calcium (Ca^{2+}), upregulation of caspase-cleaved tau (tau-C3), hyperphosphorylation of tau (ptau), and loss of cellular housekeeping or autophagy function, may contribute directly to the expression of clinical symptoms. Multiple mechanisms control these seemingly unrelated pathways. A vast amount of current literature substantiates their critical but complex role in AD pathology [1–12].

1.1. Calcium trafficking is a complex process

Ca^{2+} trafficking is a complex process. A tight functional link exists among channels located on the plasma membrane (Aβ pores; L-type calcium channels, LTCC; N-methyl D-aspartyl receptor, NMDAR), endoplasmic reticulum (ryanodine receptor, RyR; Inositol(1,4,5)P_3 receptor, InsP_3R; sarco endoplasmic reticulum calcium ATPase, SERCA), and mitochondria [13–16]. Further, presenilin proteins, involved in AD pathogenesis, are located on the ER and can leak Ca^{2+} into the cytoplasm or interact with RyR, InsP_3R, and SERCA to increase their activity [9,12,17]. A P86L polymorphism in a novel calcium channel called calcium homeostasis modulator 1 (CALHM1) can influence Aβ production by modulating amyloid precursor protein (APP) processing [11,12]. Such a complexity involving a bidirectional relationship between Aβ production and calcium homeostasis pathways

* Corresponding author at: Department of Neurology Mail Code: R&D 67 Oregon Health & Science University 3181 Sam Jackson Park Road Portland, OR 97239, USA. Tel.: +1 503 402 2968; fax: +1 503 402 2816.
E-mail address: anekondt@ohsu.edu (T.S. Anekonda).

0925-4439/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbadis.2011.08.013

presents difficult experimental challenges (Fig. 1; Sections 1 and 2). Recent publications in the triple transgenic mouse model of AD (3xTgAD) implicate roles of RyR, $InsP_3R$ and NMDAR in the production of Ca^{2+} and synaptic plasticity of hippocampal neurons [18–21], but the role of LTCC, especially of $Ca_v1.2$, in relation to Aβ, tau, and autophagy is largely unknown. Therefore, in this review, we mainly focus on $Ca_v1.2$ relationship to AD pathology.

1.2. L-type calcium channel subtype $Ca_v1.2$ is a critical target for calcium channel blockers in AD brains

Aging and Aβ consistently promote Ca^{2+} influx into neurons by way of L-type calcium channels (LTCCs). Soluble intraneuronal Aβ oligomers, soluble and insoluble Aβ fibrils can increase intracellular Ca^{2+}, impair neuronal function, and adversely affect synaptic functions in AD [13,22,23]. Ca^{2+} increase occurs through overactivation of LTCCs [24]. The uncontrolled Ca^{2+} increase can trigger the overexpression of plasma membrane LTCC subtype $Ca_v1.2$ in the hippocampus of AD brains and further exacerbate the pathogenic Ca^{2+} influx [1,25,26]. $Ca_v1.2$ is located in cell bodies and dendrites, axonal terminals, and axons of neurons, and glial processes of the hippocampus [2]. Paradoxically, $Ca_v1.2$ expression is essential for long-term potentiation (LTP) (independent of NMDAR-dependent LTP), synaptic plasticity, and spatial memory of the hippocampus [1,2]. $Ca_v1.2$KO in mice disrupts remote spatial memory, further confirming the importance of $Ca_v1.2$ [1]. $Ca_v1.3$, a closely-related LTCC subtype known to mediate the pace-making function of dopaminergic neurons in Parkinson's disease, has no known role in hippocampal memory function [27,28].

Our recent study has examined the role of $Ca_v1.2$ in response to intracellular oligomeric Aβ because such understanding is an emerging area of relevance to AD [29]. $Ca_v1.2$ channel expression is essential for long-term potentiation (LTP) independent of NMDAR-dependent LTP, synaptic plasticity, and spatial memory of the hippocampus [1–3,5,18,28]. Multiple pathways involving generation of intraneuronal or soluble extracellular Aβ can induce protein kinase A (PKA), which in turn binds to LTCC and promotes increased Ca^{2+} influx [30,31]. PKA binding to the alpha subunit of $Ca_v1.2$ in the cytoplasm causes $Ca_v1.2$ overexpression in the membrane [30,31]. PKA also promotes pathological hyperphosphorylation of tau [32,33] and suppression of autophagy function in AD [34] (Fig. 1; Sections 1 and 3).

It is important to recognize that $Ca_v1.2$-independent pathways may also directly influence Aβ production, ptau or autophagy functions. Aβ-toxicity that is independent of $Ca_v1.2$ may occur when intraneuronal Aβ directly enters mitochondria and disrupts cellular energy balance or promotes free radical production [35]. Alternatively, free radicals can be generated from APP in autophagosomal compartments and disrupt autophagy function without affecting $Ca_v1.2$ [36].

1.3. Autophagy dysfunction in AD

The emerging literature suggests that autophagy, a self-cleaning cellular housekeeping mechanism, plays an important role in AD pathology [36]. Previously, it was assumed that autophagy was inducible only in response to stress but not essential for cell function. The most recent literature, however, provides unequivocal evidence for the constitutive role of autophagy in cellular homeostasis, thus making it essential for neuronal survival [37,38]. Autophagosomal vacuoles accumulate in AD [7,36,39,40], which can lead to an accelerated accumulation of ptau and tau-C3 [41–43]. Accumulated ptau, in turn, can rupture lysosomes, causing them to prematurely release proteolytic enzymes into the cytoplasm and disrupt normal

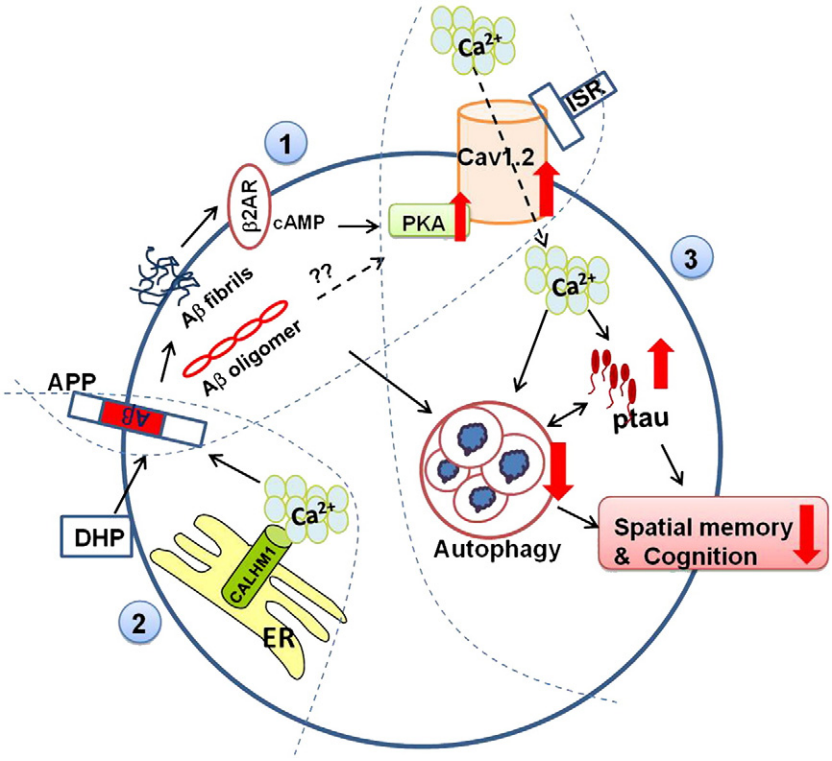

Fig. 1. A schematic overview of the Aβ–$Ca_v1.2$–ptau–autophagy pathway. Three sections of the figure are separated by dashed lines for clarity. Soluble Aβ oligomers, soluble and insoluble Aβ fibrils generated from amyloid precursor protein (APP) processing cause increased expression of $Ca_v1.2$ through beta 2 adrenergic receptor (β2AR)/cyclin AMP (cAMP) or other unknown pathways (Section 1). Aβ production can be directly modulated by mutations in the calcium homeostasis modulator 1 (CALHM1) receptor located on the endoplasmic reticulum, or in response to treatment with dihydropyridine (DHP) class of calcium channel blockers (CCBs) (Section 2). Intracellular or extracellular Aβ can cause overexpression of $Ca_v1.2$, increased influx of Ca^2, disrupted autophagy, and up-regulated ptau expression (Section 3). Ca^{2+}–calpain pathway can directly increase ptau levels or dampen autophagy function. Pathological accumulation of autophagosomal vacuoles and ptau will lead to loss of spatial memory and cognition in AD (Section 3). Isradipine (ISR) appears to block this pathway.

autophagy function leading to neuronal death by apoptosis in AD [44–46]. A heterozygous deletion of the autophagy marker beclin-1 in Tg2576 mice increases intraneuronal Aβ accumulation, extracellular Aβ deposits, and neurodegeneration [47], suggesting that autophagy plays a key protective role against AD. Autophagy function is suppressed in several mouse models including 3xTgAD [48–50]. Indeed one of the main functions of autophagy is to regulate mitochondrial function by enzymatic degradation of dysfunctional mitochondria and by clearing misfolded proteins in the cell [41,51]. Our studies in Tg2576 mice provide strong evidence for mitochondrial deposition of Aβ leading to generation of free radicals and a protective role of autophagy against AD pathology [35].

1.4. Caspase-cleaved and hyperphosphorylated tau

A considerable effort has been made in the past few decades to understand tau-associated pathology in AD [52–55]. There seems to be a general consensus among experts that: (1) hyperphosphorylated tau appears early in the disease progression and is more prone to aggregation and tangle formation than the normal tau [52,56,57]. Increased Ca^{2+} can promote ptau via calpain-dependent pathway [58,59]; (2) the presence of more 3-repeat tau conformations relative to 4-repeats can be pathogenic in AD [55,60]; and (3) caspase-cleaved tau-C3 is fibrillogenic with much greater propensity for aggregation and formation of ptau and neurofibrillary tangles [53,61,62]. The most exciting and numerous roles of tau-C3 in AD pathology are still emerging in the literature. When wild type tau is cleaved mainly by caspase-3 at Asp^{421} site, a tau-C3 is formed [56,62]. A recent study suggests that caspase activation may precede tangle formation in tau transgenic Tg4510 mice [63]. Some suggest that tau-C3 is present in tauopathies in the absence of Aβ pathology [43]. Lysosomal dysfunction may promote the increased formation of tau-C3 [44]. Thus, these emerging studies strongly suggest that the clearance pathway of tau-C3 is tightly linked to autophagy–lysosomal pathways in AD. Furthermore, tau-C3 is preferentially degraded by the macroautophagy pathway [41]. Ca^{2+} toxicity or overexpression of $Ca_v1.2$ can also lead to mislocalization of tau into somatodendritic regions instead of normal axonal localization [64]. In somatodendritic regions, tau can damage microtubules and spines and become ptau [64].

These studies together provide a strong evidence for complex roles of Aβ, $Ca_v1.2$, Ca_i^{2+}, tau-C3, ptau, autophagy–lysosomal dysfunction in orchestrating AD pathology. We call this nexus of toxicity in short: Aβ–$Ca_v1.2$–ptau–autophagy pathway.

2. Calcium channel blockers to break the nexus of toxicity

Usefulness of LTCC blockers (CCBs) against AD pathology is controversial. Epidemiological studies suggest that CCBs prevent [65] or slow the rate of progression of AD [66,67]. A large clinical trial with nimodipine did not show significant benefits from the primary outcome measures but has shown moderate benefits for treatment of AD in the secondary outcome measures [68,69]. In a recent study, however, nimodipine selectively stimulated the secretion of $Aβ_{1–42}$ *in vitro* and in the plasma of Tg2576 mouse model of AD, questioning the usefulness of this CCB for AD [70]. Several clinical studies have suggested that CCBs used as antihypertensive drugs may prevent cognitive decline in hypertensive subjects [71–74], but none has demonstrated a role for CCBs in AD *per se*. Two dihydropyridine CCBs were among the eight FDA-approved drugs selected from 485 small biomolecules screened for their ability to induce autophagy without causing toxic effects in human neuroglioma H4 cells [75]. Further, dihydropyridine-based derivatives act as potent activators of anti-aging neuroprotective protein sirtuin 1 [76], which appears to regulate autophagy function [77].

Two recent *in vitro* studies have shown usefulness of DHP CCBs on Aβ production and $Aβ_{1–42}$ transcytosis across an *in vitro* blood–brain barrier (BBB) created by an apical "blood" and basolateral "brain" layers of human brain microvascular endothelial cells [78,79]. Nilvadipine, nitrendipine, and amlodipine reduced Aβ production in 7W Chinese hamster ovary (CHO) cells, which have been stably transfected with human APP751 [79]. This study further shows an improved Aβ clearance by nilvadipine and nitrendipine, and an improved explorative activity for animals treated with nilvadipine in a transgenic animal model of AD (Tg PS1/APPsw) [79]. The BBB transcytosis of $Aβ_{1–42}$ also increased for several of DHP CCBs including nitrendipine, nicardipine, nimodipine, and nilvadipine [78]. In their study, isradipine had no effect on $Aβ_{1–42}$ transcytosis. Furthermore, CCBs (nitrendipine, cilnidipine, nilvadipine) promoted $Aβ_{1–42}$ clearance across the BBB in wild type mice; and in animals treated with human $Aβ_{1–42}$, nilvadipine improved the cognitive functions of the animals in Morris water maze test [78]. These studies clearly suggest that DHP CCBs possess non-channeling functions that are independent of their calcium channel blocking ability, suggesting a need for thorough validation of CCBs for AD.

2.1. Why isradipine a suitable CCB for treatment of AD?

Emerging studies in models of Parkinson's disease (PD) show neuroprotection by isradipine, an FDA-approved dihydropyridine class of CCB for hypertension [27,80–82]. In these studies, isradipine blocks LTCC subtype $Ca_v1.3$ function in dopaminergic neuron of the substantia nigra and modulates autonomous pacemaking function [27,82,83]. Isradipine provides protection against stroke and brain ischemia in rat models for hypertension [84,85]. Early studies focusing on binding properties of [^{3}H]isradipine in AD and control brains suggest that the hippocampal CA1 region experiences greater cell loss in response to increased expression of LTCCs in AD brains relative to control brains [86,87], suggesting that isradipine treatment is likely to modulate the over-expressing LTCCs in the CA1 region and preserve the hippocampal function. Isradipine can also attenuate over active LTCC function as well as oxidative stress-induced apoptotic cell death in hypobaric hypoxia model of rats and preserve their memory function [88]. Our preliminary studies have clearly shown the superior effects of isradipine over nimodipine *in vitro* [29]. We also predict that the brain bioavailability of therapeutic doses of isradipine is superior to that of nimodipine, as bioavailability studies in animals generally support this assertion [89,90].

A recent isradipine safety and tolerability study in PD provides valuable guidelines on how to evaluate the neuroprotective effects of isradipine in clinical trials [80]. In this study, subjects (average age = ~59 years) with early PD were treated with increasing doses of controlled release isradipine (5–20 mg daily doses) over a period of eight weeks. There was a dose-dependent tolerability for isradipine (94% for 5 mg; 87% for 10 mg; 68% for 15 mg; 52% for 20 mg). Isradipine did not show any significant impact on blood pressure or motor disability, but leg edema and dizziness were the two frequent adverse symptoms commonly observed in this study, leading to a conclusion that 10 mg daily dose of isradipine was a safe treatment [80]. In a Danish case–control study (average age = 72 years), the effect of DHP class of CCBs was evaluated retrospectively for their neuroprotective effects on PD [27]. The commonly prescribed centrally-acting DHP CCBs (nimodipine, isradipine, nitrendipine, nifedipine), as opposed to non-DHP class of CCBs, provided up to 27% risk reduction for PD, irrespective of treatment length and duration [27]. These studies appear to suggest that careful clinical trials using isradipine in older patients are highly feasible, safe, and unlikely to cause adverse effects on the memory-related function of $Ca_v1.2$.

2.1.1. In vitro studies

Our recent publication is the first such report to show neuroprotection of isradipine against Aβ-induced Ca^{2+} toxicity in human neuroblastoma/MC65 cells [29]. MC65 cells that are stably transfected

with the APP–C99 gene conditionally express a fusion protein fragment of the amino-17 and carboxyl-99 residues [91,92]. APP–C99 gene expression in these cells is controlled by a tetracycline-responsive promoter whose activity is repressed in the presence of tetracycline or induced by withdrawing tetracycline from the growth medium. Removal of tetracycline leads to expression of the C-terminal APP fragment and subsequent processing of this fragment into Aβ monomers followed by accumulation of intracellular Aβ oligomers, and precipitous cell death by about 72 h. In this cell culture model we tested the role of $Ca_v1.2$ expression on Ca_i^{2+} influx and cell survival as well as protective effects of four CCBs (diltiazem, verapamil, nimodipine, isradipine) against Ca_i^{2+}-induced toxicity. Isradipine was the most potent of the four CCBs tested [29]. This study suggests that intracellular Aβ oligomers trigger increased intracellular Ca^{2+} influx primarily through $Ca_v1.2$ channels. None of these CCBs prevented the formation of intracellular oligomers. Isradipine provided protection against Ca^{2+} toxicity by both blocking calcium influx and suppressing $Ca_v1.2$ expression downstream of Aβ formation [29]. Our study results also indicate that Ca^{2+} levels are tightly controlled and cells respond to small variations in Ca^{2+} and this response is sensitive to small concentrations of isradipine [29].

2.1.2. In vivo studies

We also tested isradipine for its neuroprotective functions in four evolutionarily divergent species including models for AD: human neuroblastoma/MC65 cells, transgenic *Drosophila*, *Monduca*, and 3xTgAD mice [93]. In transgenic *Drosophila* model, 250 μM isradipine increased the survival of flies from 6.5% to 12% ($p<0.5$) against APP_{695} toxicity [93]. In moth/*Monduca sexta* experiments, embryonic culture preparations were exposed to exogenous $Aβ_{1-42}$ peptide to determine the toxic effects of this peptide on neuronal development and growth in the presence or absence of 10 μM isradipine [93]. Treatment with $Aβ_{1-42}$ induces concentration-dependent perturbations in the extent of migration and outgrowth of enteric nervous system. A simultaneous treatment with isradipine prevents the deleterious effects of Aβ [93]. Our studies further show that subcutaneous administration of isradipine (3 μg/g/day; 60-day release) to 3xTgAD mice was well-tolerated and isradipine became available to the brain [29,93]. The 3xTgAD mouse model, which harbors PS1 (M146V), APPswe and tau (P301L) transgenes, develops age-dependent and region-specific Aβ and tau aggregations in the cortex and hippocampus that closely mimics the pathology found in human AD [94,95]. A small cohort of 17-month-old female 3xTgAD mice (an age well after the appearance of AD pathology) was implanted with carrier-bound isradipine pellets ($n=3$, 3 mg/kg/day, 60-day release, Innovative Research of America) or placebo control pellets ($n=4$). Isradipine was well-tolerated as evidenced by average weekly body weights (an indirect measurement of toxicity), which were the same for vehicle and isradipine-treated animals. Isradipine treatment also showed lowering trend for insoluble $Aβ_{1-40}$ and $Aβ_{1-42}$, no effect on soluble $Aβ_{1-40}$, and undetectable soluble $Aβ_{1-42}$ [93]. Histological Aβ burden in these animals reduced non-significantly in the hippocampus and isradipine significantly lowered IHC-detectable ptau burden in the hippocampus [93]. In a second cohort of 22-month-old female 3xTgAD ($N=8$) and age-matched wild type ($N=8$) mice, a similar isradipine or vehicle pellet implantation showed significantly upregulated LC3B protein, a marker for late-stage autophagy (unpublished results) in isradipine-treated mice. These animal studies together strongly suggest that protective functions of isradipine are similarly conserved across evolutionarily divergent species and these functions are mutually synergistic.

2.2. LTCC blocking with isradipine: implications for future research

Our novel findings show that isradipine can modulate AD pathology by influencing Aβ production upstream as well as components of the $Ca_v1.2$–ptau–autophagy pathway. This will have much larger implications in developing a unique treatment strategy for AD. Isradipine appears to bind strongly to $Ca_v1.2$ in the hippocampus and perhaps short-circuits the downstream pathway [3,96,97] (Fig. 1; Section 3). Mechanisms that promote the clearance of rapidly cluttering autophagosomal vacuoles in AD brains have been elusive for the past several decades, and they now appear to be an important bottleneck in moving forward with effective treatment strategies for AD. Fortunately, calcium channel blockers are emerging as modulators of autophagy function [75,98]. We believe that an approach to use CCB to short-circuit the entire downstream pathway can overcome current hurdles in the clinical treatment of AD. We argue that isradipine is an appropriate candidate for future studies, as it appears to possess additional functions capable of modulating both upstream and downstream protective functions besides its calcium channel blocking ability.

Calcium channel blocking will have many implications for future research and clinical trials: (1) suppression of $Ca_v1.2$ expression through β2AR/cAMP/PKA or mitochondrial PKA-dependent pathways can defuse the upstream toxic effects of Aβ or reactive oxygen species [30,31]. (2) PKA-dependent suppression of LC3 function in autophagy can be neutralized by blocking $Ca_v1.2$ [99,100]. (3) Attenuated Ca^{2+}-dependent calpain levels can downregulate ptau and caspase-cleaved tau production. It is possible that LTCC blocking may provide effective protection strategies for Parkinson's disease, Huntington's disease, ALS, axotomy, brain ischemia, and stroke, where autophagy or isradipine is known to play key protective roles. It is important to recognize that there may be some risk in modulating $Ca_v1.2$ expression specifically in the hippocampal CA1 pyramidal neurons, since these receptors also directly influence long-term potentiation, synaptic plasticity, and spatial memory function [3,5,28,101]. However, several lines of evidence discussed at the top of this section suggest that isradipine predominantly modulates $Ca_v1.2$ rather than adversely effecting the memory function associated with normal function of $Ca_v1.2$ [27,80,86].

3. Bioavailability of calcium channel blockers

In the past three decades, several bioanalytical methods (radioactive labeling, gas chromatography, high pressure liquid chromatography) have been developed for assessing isradipine pharmacokinetics and pharmacodynamics in animal and human tissues [6,86,89,90,102,103]. The most important discovery from these early methods is that isradipine undergoes extensive first-pass metabolism and nearly 90% of orally administered isradipine is absorbed in the digestive tract, limiting its bioavailability to about 17–28% in plasma. For an immediate release (IR) formulation of isradipine, a sharp peak concentration occurs in plasma about 1.5–2 h after administration with a terminal half-life of 8 h [89]. Sustained-release (SR) formulations show superior bioavailability with an initial lag period for 2–3 h and then a slow increase in concentration reaching a plateau between 7 and 18 h after dosing. Thus, SR formulation shows a lower maximal plasma concentration (C_{max}) and an extended maximal tissue concentration (T_{max}) as well as extended mean elimination half-life ($T_{½}$) compared to IR formulation [89]. However, many of the early methods showed low sensitivity, longer analytical time, and involved pre-column derivatization protocols affected by interferences, making them prohibitively expensive for large-scale rapid screening of clinical samples. Recently, a liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) has been developed as sensitive, accurate, and rapid method for assessment of isradipine bioavailability in the plasma samples from healthy volunteers [104]. This method showed an excellent linearity between 10 and 5000 pg/ml of isradipine in plasma, with the lower limit of quantitation (LLOQ) at 10 pg/ml plasma and a short retention time [104]. We have recently applied this method successfully to a 3xTgAD mice [29]. In our studies with 3xTgAD mice, we detected 33 ± 7 ng/ml of isradipine in the plasma and

47 ± 1 ng/g brain tissue of the animals implanted with carrier-bound isradipine pellets (3 μg/g/day, 60-day release, Innovative Research of America, Sarasota, FL) and none in animals implanted with placebo control pellets [29].

To be able to apply the above method to AD patients, we need to establish relationships between plasma and brain bioavailability of isradipine first in animal models of AD. This approach needs some practical considerations. First, there is a possibility that the plasma-to-brain relationship for isradipine may be a non-linear function. Because isradipine can easily cross the blood–brain barrier [105], we expect isradipine will reach the brain readily. Thus the non-linearity argument may be somewhat muted. Second, there is a possibility that T_{max} and $T_{½}$ for isradipine may differ from mouse to human. Interestingly, cross-species pharmacokinetics appears to be remarkably comparable between mouse and human, including for isradipine [89,103,106]. So, these parameters are expected to remain similar in mouse and human brains; thus, information obtained on mouse models can be easily translatable to humans. Third, transgenic animal models do not fully account for the genetic heterogeneity prevalent in the late-onset AD population. This is a serious issue and one way to maximize heterogeneity in animal populations is by using different genotypes/animal models and age groups. Alternatively, pharmacokinetic measures such as area under curve (AUC) can be determined for plasma and brain to ensure a greater accuracy. Such an approach will require multiple time points of measurements after isradipine dosing and will increase the number of animals required for the study. In live humans, brain function or cerebrospinal fluid levels rather that brain levels of isradipine can be modeled with plasma levels of isradipine to develop clinically useful predictive functions.

4. Conclusions

Despite their long-standing presence and well-defined safety records, the usefulness of CCBs for Alzheimer's disease has been unclear. The reluctance to use CCBs to AD may be attributed to the initial failure of nimodipine in an AD clinical trial. However, recent experimental evidence presented in this review demonstrates that CCBs such as isradipine possess multiple beneficial effects besides their primary L-type calcium blocking ability. Rigorous, well-designed pre-clinical and clinical studies are expected to provide proof-of-concept on the effectiveness of CCBs for treatment of Alzheimer's disease.

Acknowledgements

This work was supported by NIH/NEI 5R21EY018708-02 (TSA), Department of Veterans Affairs Merit Review Grant (JFQ), and NIA-R21AG027445A (JFQ).

References

[1] J.A. White, B.C. McKinney, M.C. John, P.A. Powers, T.J. Kamp, G.G. Murphy, Conditional forebrain deletion of the L-type calcium channel $Ca_V1.2$ disrupts remote spatial memories in mice, Learn. Mem. 15 (2008) 1–5.
[2] A.L. Tippens, J.F. Pare, N. Langwieser, S. Moosmang, T.A. Milner, Y. Smith, A. Lee, Ultrastructural evidence for pre- and postsynaptic localization of $Ca_v1.2$ L-type Ca^{2+} channels in the rat hippocampus, J. Comp. Neurol. 506 (2008) 569–583.
[3] L. Lacinova, S. Moosmang, N. Langwieser, F. Hofmann, T. Kleppisch, $Ca_v1.2$ calcium channels modulate the spiking pattern of hippocampal pyramidal cells, Life Sci. 82 (2008) 41–49.
[4] S.F. Oliveria, M.L. Dell'Acqua, W.A. Sather, AKAP79/150 anchoring of calcineurin controls neuronal L-type Ca^{2+} channel activity and nuclear signaling, Neuron 55 (2007) 261–275.
[5] S. Moosmang, N. Haider, N. Klugbauer, H. Adelsberger, N. Langwieser, J. Muller, M. Stiess, E. Marais, V. Schulla, L. Lacinova, S. Goebbels, K.A. Nave, D.R. Storm, F. Hofmann, T. Kleppisch, Role of hippocampal $Ca_v1.2$ Ca^{2+} channels in NMDA receptor-independent synaptic plasticity and spatial memory, J. Neurosci. 25 (2005) 9883–9892.
[6] S. Uchida, S. Yamada, K. Nagai, Y. Deguchi, R. Kimura, Brain pharmacokinetics and in vivo receptor binding of 1,4-dihydropyridine calcium channel antagonists, Life Sci. 61 (1997) 2083–2090.
[7] R.A. Nixon, J. Wegiel, A. Kumar, W.H. Yu, C. Peterhoff, A. Cataldo, A.M. Cuervo, Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study, J. Neuropathol. Exp. Neurol. 64 (2005) 113–122.
[8] M.P. Mattson, Pathways towards and away from Alzheimer's disease, Nature 430 (2004) 631–639.
[9] K.H. Cheung, D. Shineman, M. Muller, C. Cardenas, L. Mei, J. Yang, T. Tomita, T. Iwatsubo, V.M. Lee, J.K. Foskett, Mechanism of Ca^{2+} disruption in Alzheimer's disease by presenilin regulation of InsP3 receptor channel gating, Neuron 58 (2008) 871–883.
[10] F.M. LaFerla, Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease, Nat. Rev. Neurosci. 3 (2002) 862–872.
[11] U. Dreses-Werringloer, J.C. Lambert, V. Vingtdeux, H. Zhao, H. Vais, A. Siebert, A. Jain, J. Koppel, A. Rovelet-Lecrux, D. Hannequin, F. Pasquier, D. Galimberti, E. Scarpini, D. Mann, C. Lendon, D. Campion, P. Amouyel, P. Davies, J.K. Foskett, F. Campagne, P. Marambaud, A polymorphism in CALHM1 influences Ca^{2+} homeostasis, Abeta levels, and Alzheimer's disease risk, Cell 133 (2008) 1149–1161.
[12] K.N. Green, F.M. LaFerla, Linking calcium to Abeta and Alzheimer's disease, Neuron 59 (2008) 190–194.
[13] I. Bezprozvanny, M.P. Mattson, Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease, Trends Neurosci. 31 (2008) 454–463.
[14] N. Arispe, H.B. Pollard, E. Rojas, beta-Amyloid Ca(2+)-channel hypothesis for neuronal death in Alzheimer disease, Mol. Cell. Biochem. 140 (1994) 119–125.
[15] Q. Guo, K. Furukawa, B.L. Sopher, D.G. Pham, J. Xie, N. Robinson, G.M. Martin, M.P. Mattson, Alzheimer's PS-1 mutation perturbs calcium homeostasis and sensitizes PC12 cells to death induced by amyloid beta-peptide, Neuroreport 8 (1996) 379–383.
[16] K.N. Green, A. Demuro, Y. Akbari, B.D. Hitt, I.F. Smith, I. Parker, F.M. LaFerla, SERCA pump activity is physiologically regulated by presenilin and regulates amyloid beta production, J. Cell Biol. 181 (2008) 1107–1116.
[17] S.L. Chan, M. Mayne, C.P. Holden, J.D. Geiger, M.P. Mattson, Presenilin-1 mutations increase levels of ryanodine receptors and calcium release in PC12 cells and cortical neurons, J. Biol. Chem. 275 (2000) 18195–18200.
[18] J.R. Lopez, A. Lyckman, S. Oddo, F.M. Laferla, H.W. Querfurth, A. Shtifman, Increased intraneuronal resting $[Ca^{2+}]$ in adult Alzheimer's disease mice, J. Neurochem. 105 (2008) 262–271.
[19] S. Chakroborty, I. Goussakov, M.B. Miller, G.E. Stutzmann, Deviant ryanodine receptor-mediated calcium release resets synaptic homeostasis in presymptomatic 3xTg-AD mice, J. Neurosci. 29 (2009) 9458–9470.
[20] I. Goussakov, M.B. Miller, G.E. Stutzmann, NMDA-mediated Ca(2+) influx drives aberrant ryanodine receptor activation in dendrites of young Alzheimer's disease mice, J. Neurosci. 30 (2010) 12128–12137.
[21] I.F. Smith, B. Hitt, K.N. Green, S. Oddo, F.M. LaFerla, Enhanced caffeine-induced Ca^{2+} release in the 3xTg-AD mouse model of Alzheimer's disease, J. Neurochem. 94 (2005) 1711–1718.
[22] M.P. Mattson, Calcium and neurodegeneration, Aging Cell 6 (2007) 337–350.
[23] C. Haass, D.J. Selkoe, Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide, Nat. Rev. Mol. Cell Biol. 8 (2007) 101–112.
[24] K. Ueda, S. Shinohara, T. Yagami, K. Asakura, K. Kawasaki, Amyloid beta protein potentiates Ca^{2+} influx through L-type voltage-sensitive Ca^{2+} channels: a possible involvement of free radicals, J. Neurochem. 68 (1997) 265–271.
[25] M. Willis, W.A. Kaufmann, G. Wietzorrek, B. Hutter-Paier, S. Moosmang, C. Humpel, F. Hofmann, M. Windisch, H.G. Knaus, J. Marksteiner, L-type calcium channel Ca_V 1.2 in transgenic mice overexpressing human AbetaPP751 with the London (V717I) and Swedish (K670M/N671L) mutations, J. Alzheimers Dis. 20 (2010) 1167–1180.
[26] N. Langwieser, C.J. Christel, T. Kleppisch, F. Hofmann, C.T. Wotjak, S. Moosmang, Homeostatic switch in hebbian plasticity and fear learning after sustained loss of $Ca_v1.2$ calcium channels, J. Neurosci. 30 (2010) 8367–8375.
[27] B. Ritz, S.L. Rhodes, L. Qian, E. Schernhammer, J.H. Olsen, S. Friis, L-type calcium channel blockers and Parkinson disease in Denmark, Ann. Neurol. 67 (2010) 600–606.
[28] H. Zhang, Y. Fu, C. Altier, J. Platzer, D.J. Surmeier, I. Bezprozvanny, Ca1.2 and $Ca_V1.3$ neuronal L-type calcium channels: differential targeting and signaling to pCREB, Eur. J. Neurosci. 23 (2006) 2297–2310.
[29] T.S. Anekonda, J.F. Quinn, C. Harris, K. Frahler, T.L. Wadsworth, R.L. Woltjer, L-type voltage-gated calcium channel blockade with isradipine as a therapeutic strategy for Alzheimer's disease, Neurobiol. Dis. 41 (2011) 62–70.
[30] D. Wang, G. Govindaiah, R. Liu, V. De Arcangelis, C.L. Cox, Y.K. Xiang, Binding of amyloid beta peptide to beta2 adrenergic receptor induces PKA-dependent AMPA receptor hyperactivity, FASEB J. 24 (2010) 3511–3521.
[31] D.D. Hall, M.A. Davare, M. Shi, M.L. Allen, M. Weisenhaus, G.S. McKnight, J.W. Hell, Critical role of cAMP-dependent protein kinase anchoring to the L-type calcium channel $Ca_v1.2$ via A-kinase anchor protein 150 in neurons, Biochemistry 46 (2007) 1635–1646.
[32] H. Yamamoto, Y. Hiragami, M. Murayama, K. Ishizuka, M. Kawahara, A. Takashima, Phosphorylation of tau at serine 416 by Ca^{2+}/calmodulin-dependent protein kinase II in neuronal soma in brain, J. Neurochem. 94 (2005) 1438–1447.
[33] C.W. Scott, R.C. Spreen, J.L. Herman, F.P. Chow, M.D. Davison, J. Young, C.B. Caputo, Phosphorylation of recombinant tau by cAMP-dependent protein kinase. Identification of phosphorylation sites and effect on microtubule assembly, J. Biol. Chem. 268 (1993) 1166–1173.
[34] N.S. Honson, J. Kuret, Tau aggregation and toxicity in tauopathic neurodegenerative diseases, J. Alzheimers Dis. 14 (2008) 417–422.
[35] M. Manczak, T.S. Anekonda, E. Henson, B.S. Park, J. Quinn, P.H. Reddy, Mitochondria are a direct site of Abeta accumulation in Alzheimer's disease neurons:

implications for free radical generation and oxidative damage in disease progression, Hum. Mol. Genet. 15 (2006) 1437–1449.
[36] R.A. Nixon, Autophagy, amyloidogenesis and Alzheimer disease, J. Cell Sci. 120 (2007) 4081–4091.
[37] T. Hara, K. Nakamura, M. Matsui, A. Yamamoto, Y. Nakahara, R. Suzuki-Migishima, M. Yokoyama, K. Mishima, I. Saito, H. Okano, N. Mizushima, Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice, Nature 441 (2006) 885–889.
[38] M. Komatsu, S. Waguri, T. Chiba, S. Murata, J. Iwata, I. Tanida, T. Ueno, M. Koike, Y. Uchiyama, E. Kominami, K. Tanaka, Loss of autophagy in the central nervous system causes neurodegeneration in mice, Nature 441 (2006) 880–884.
[39] W.H. Yu, A.M. Cuervo, A. Kumar, C.M. Peterhoff, S.D. Schmidt, J.H. Lee, P.S. Mohan, M. Mercken, M.R. Farmery, L.O. Tjernberg, Y. Jiang, K. Duff, Y. Uchiyama, J. Naslund, P.M. Mathews, A.M. Cataldo, R.A. Nixon, Macroautophagy—a novel beta-amyloid peptide-generating pathway activated in Alzheimer's disease, J. Cell Biol. 171 (2005) 87–98.
[40] R.A. Nixon, Autophagy in neurodegenerative disease: friend, foe or turncoat? Trends Neurosci. 29 (2006) 528–535.
[41] P.J. Dolan, G.V. Johnson, A caspase cleaved form of tau is preferentially degraded through the autophagy pathway, J. Biol. Chem. 285 (2010) 21978–21987.
[42] J.H. Lee, W.H. Yu, A. Kumar, S. Lee, P.S. Mohan, C.M. Peterhoff, D.M. Wolfe, M. Martinez-Vicente, A.C. Massey, G. Sovak, Y. Uchiyama, D. Westaway, A.M. Cuervo, R.A. Nixon, Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations, Cell 141 (2010) 1146–1158.
[43] C.W. Cotman, W.W. Poon, R.A. Rissman, M. Blurton-Jones, The role of caspase cleavage of tau in Alzheimer disease neuropathology, J. Neuropathol. Exp. Neurol. 64 (2005) 104–112.
[44] V. Khurana, I. Elson-Schwab, T.A. Fulga, K.A. Sharp, C.A. Loewen, E. Mulkearns, J. Tyynela, C.R. Scherzer, M.B. Feany, Lysosomal dysfunction promotes cleavage and neurotoxicity of tau in vivo, PLoS Genet. 6 (2010) e1001026.
[45] A.M. Cataldo, R.A. Nixon, Enzymatically active lysosomal proteases are associated with amyloid deposits in Alzheimer brain, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 3861–3865.
[46] T. Hamano, T.F. Gendron, E. Causevic, S.H. Yen, W.L. Lin, C. Isidoro, M. Deture, L.W. Ko, Autophagic–lysosomal perturbation enhances tau aggregation in transfectants with induced wild-type tau expression, Eur. J. Neurosci. 27 (2008) 1119–1130.
[47] F. Pickford, E. Masliah, M. Britschgi, K. Lucin, R. Narasimhan, P.A. Jaeger, S. Small, B. Spencer, E. Rockenstein, B. Levine, T. Wyss-Coray, The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice, J. Clin. Invest. 118 (2008) 2190–2199.
[48] A. Caccamo, S. Majumder, A. Richardson, R. Strong, S. Oddo, Molecular interplay between mammalian target of rapamycin (mTOR), amyloid-beta, and Tau: effects on cognitive impairments, J. Biol. Chem. 285 (2010) 13107–13120.
[49] T. Ma, C.A. Hoeffer, E. Capetillo-Zarate, F. Yu, H. Wong, M.T. Lin, D. Tampellini, E. Klann, R.D. Blitzer, G.K. Gouras, Dysregulation of the mTOR pathway mediates impairment of synaptic plasticity in a mouse model of Alzheimer's disease, PLoS ONE 5 (2010) e12845.
[50] P. Spilman, N. Podlutskaya, M.J. Hart, J. Debnath, O. Gorostiza, D. Bredesen, A. Richardson, R. Strong, V. Galvan, Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-beta levels in a mouse model of Alzheimer's disease, PLoS ONE 5 (2010) e9979.
[51] S.M. Cardoso, C.F. Pereira, P.I. Moreira, D.M. Arduino, A.R. Esteves, C.R. Oliveira, Mitochondrial control of autophagic lysosomal pathway in Alzheimer's disease, Exp. Neurol. 223 (2009) 294–298.
[52] H. Braak, E. Braak, M. Strothjohann, Abnormally phosphorylated tau protein related to the formation of neurofibrillary tangles and neuropil threads in the cerebral cortex of sheep and goat, Neurosci. Lett. 171 (1994) 1–4.
[53] P. Koson, N. Zilka, A. Kovac, B. Kovacech, M. Korenova, P. Filipcik, M. Novak, Truncated tau expression levels determine life span of a rat model of tauopathy without causing neuronal loss or correlating with terminal neurofibrillary tangle load, Eur. J. Neurosci. 28 (2008) 239–246.
[54] Q. Zhang, X. Zhang, A. Sun, Truncated tau at D421 is associated with neurodegeneration and tangle formation in the brain of Alzheimer transgenic models, Acta Neuropathol. 117 (2009) 687–697.
[55] A. Siddiqua, M. Margittai, Three- and four-repeat Tau coassemble into heterogeneous filaments: an implication for Alzheimer disease, J. Biol. Chem. 285 (2010) 37920–37926.
[56] M. Zilkova, N. Zilka, A. Kovac, B. Kovacech, R. Skrabana, M. Skrabanova, M. Novak, Hyperphosphorylated truncated protein tau induces caspase-3 independent apoptosis-like pathway in the Alzheimer's disease cellular model, J. Alzheimers Dis. 23 (2011) 161–169.
[57] A.C. Alonso, I. Grundke-Iqbal, K. Iqbal, Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules, Nat. Med. 2 (1996) 783–787.
[58] K.S. Hung, S.L. Hwang, C.L. Liang, Y.J. Chen, T.H. Lee, J.K. Liu, S.L. Howng, C.H. Wang, Calpain inhibitor inhibits p35-p25-Cdk5 activation, decreases tau hyperphosphorylation, and improves neurological function after spinal cord hemisection in rats, J. Neuropathol. Exp. Neurol. 64 (2005) 15–26.
[59] L.S. Yang, H. Ksiezak-Reding, Calpain-induced proteolysis of normal human tau and tau associated with paired helical filaments, Eur. J. Biochem. 233 (1995) 9–17.
[60] Y. Fujino, D.S. Wang, N. Thomas, M. Espinoza, P. Davies, D.W. Dickson, Increased frequency of argyrophilic grain disease in Alzheimer disease with 4R tau-specific immunohistochemistry, J. Neuropathol. Exp. Neurol. 64 (2005) 209–214.
[61] R.A. Quintanilla, T.A. Matthews-Roberson, P.J. Dolan, G.V. Johnson, Caspase-cleaved tau expression induces mitochondrial dysfunction in immortalized cortical neurons: implications for the pathogenesis of Alzheimer disease, J. Biol. Chem. 284 (2009) 18754–18766.
[62] N. Zilka, P. Filipcik, P. Koson, L. Fialova, R. Skrabana, M. Zilkova, G. Rolkova, E. Kontsekova, M. Novak, Truncated tau from sporadic Alzheimer's disease suffices to drive neurofibrillary degeneration in vivo, FEBS Lett. 580 (2006) 3582–3588.
[63] A. de Calignon, L.M. Fox, R. Pitstick, G.A. Carlson, B.J. Bacskai, T.L. Spires-Jones, B.T. Hyman, Caspase activation precedes and leads to tangles, Nature 464 (2010) 1201–1204.
[64] H. Zempel, E. Thies, E. Mandelkow, E.M. Mandelkow, Abeta oligomers cause localized Ca(2+) elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines, J. Neurosci. 30 (2010) 11938–11950.
[65] F. Forette, M.L. Seux, J.A. Staessen, L. Thijs, W.H. Birkenhager, M.R. Babarskiene, S. Babeanu, A. Bossini, B. Gil-Extremera, X. Girerd, T. Laks, E. Lilov, V. Moisseyev, J. Tuomilehto, H. Vanhanen, J. Webster, Y. Yodfat, R. Fagard, Prevention of dementia in randomised double-blind placebo-controlled Systolic Hypertension in Europe (Syst-Eur) trial, Lancet 352 (1998) 1347–1351.
[66] J. Fritze, J. Walden, Clinical findings with nimodipine in dementia: test of the calcium hypothesis, J. Neural. Transm. Suppl. 46 (1995) 439–453.
[67] G.D. Tollefson, Short-term effects of the calcium channel blocker nimodipine (Bay-e-9736) in the management of primary degenerative dementia, Biol. Psychiatry 27 (1990) 1133–1142.
[68] B.J. Lopez-Arrieta, Nimodipine for primary degenerative, mixed and vascular dementia, Cochrane Database of Systematic Reviews, 2002 article no CD000147.
[69] F. Morich, F. Bieber, J.M. Lewis, L. Kaiser, N.R. Cutler, J.I. Escobar, J. Willmer, R.C. Petersen, B. Reisberg, Nimodipine in the treatment of probably Alzheimer's disease results of two multicentre trials, Clin. Drug Invest. 11 (1996) 185–196.
[70] F. Facchinetti, C. Fasolato, E. Del Giudice, A. Burgo, S. Furegato, M. Fusco, E. Basso, R. Seraglia, A. D'Arrigo, A. Leon, Nimodipine selectively stimulates beta-amyloid 1–42 secretion by a mechanism independent of calcium influx blockage, Neurobiol. Aging 27 (2006) 218–227.
[71] K.M. Bellew, J.G. Pigeon, P.E. Stang, W. Fleischman, R.M. Gardner, W.W. Baker, Hypertension and the rate of cognitive decline in patients with dementia of the Alzheimer type, Alzheimer Dis. Assoc. Disord. 18 (2004) 208–213.
[72] H. Hanyu, K. Hirao, S. Shimizu, T. Sato, A. Kiuchi, T. Iwamoto, Nilvadipine prevents cognitive decline of patients with mild cognitive impairment, Int. J. Geriatr. Psychiatry 22 (2007) 1264–1266.
[73] A.S. Khachaturian, P.P. Zandi, C.G. Lyketsos, K.M. Hayden, I. Skoog, M.C. Norton, J.T. Tschanz, L.S. Mayer, K.A. Welsh-Bohmer, J.C. Breitner, Antihypertensive medication use and incident Alzheimer disease: the Cache County Study, Arch. Neurol. 63 (2006) 686–692.
[74] S. Yasar, M. Corrada, R. Brookmeyer, C. Kawas, Calcium channel blockers and risk of AD: the Baltimore Longitudinal Study of Aging, Neurobiol. Aging 26 (2005) 157–163.
[75] L. Zhang, J. Yu, H. Pan, P. Hu, Y. Hao, W. Cai, H. Zhu, A.D. Yu, X. Xie, D. Ma, J. Yuan, Small molecule regulators of autophagy identified by an image-based high-throughput screen, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 19023–19028.
[76] A. Mai, S. Valente, S. Meade, V. Carafa, M. Tardugno, A. Nebbioso, A. Galmozzi, N. Mitro, E. De Fabiani, L. Altucci, A. Kazantsev, Study of 1,4-dihydropyridine structural scaffold: discovery of novel sirtuin activators and inhibitors, J. Med. Chem. 52 (2009) 5496–5504.
[77] I.H. Lee, L. Cao, R. Mostoslavsky, D.B. Lombard, J. Liu, N.E. Bruns, M. Tsokos, F.W. Alt, T. Finkel, A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 3374–3379.
[78] C. Bachmeier, D. Beaulieu-Abdelahad, M. Mullan, D. Paris, Selective dihydropyridine compounds facilitate the clearance of beta-amyloid across the blood–brain barrier, Eur. J. Pharmacol. 659 (2011) 124–129.
[79] D. Paris, C. Bachmeier, N. Patel, A. Quadros, C.H. Volmar, V. Laporte, J. Ganey, D. Beaulieu-Abdelahad, G. Ait-Ghezala, F. Crawford, M.J. Mullan, Selective antihypertensive dihydropyridines lower abeta accumulation by targeting both the production and the clearance of abeta across the blood–brain barrier, Mol. Med. 17 (2011) 149–162.
[80] T. Simuni, E. Borushko, M.J. Avram, S. Miskevics, A. Martel, C. Zadikoff, A. Videnovic, F.M. Weaver, K. Williams, D.J. Surmeier, Tolerability of isradipine in early Parkinson's disease: a pilot dose escalation study, Mov. Dis. 25 (2010) 2863–2866.
[81] C.C. Chang, S. Cao, S. Kang, L. Kai, X. Tian, P. Pandey, S.F. Dunne, C.H. Luan, D.J. Surmeier, R.B. Silverman, Antagonism of 4-substituted 1,4-dihydropyridine-3,5-dicarboxylates toward voltage-dependent L-type Ca^{2+} channels Ca V 1.3 and Ca V 1.2, Bioorg. Med. Chem. 18 (2010) 3147–3158.
[82] C.S. Chan, T.S. Gertler, D.J. Surmeier, A molecular basis for the increased vulnerability of substantia nigra dopamine neurons in aging and Parkinson's disease, Mov. Disord. 25 (Suppl. 1) (2010) S63–70.
[83] A. Singh, M. Gebhart, R. Fritsch, M.J. Sinnegger-Brauns, C. Poggiani, J.C. Hoda, J. Engel, C. Romanin, J. Striessnig, A. Koschak, Modulation of voltage- and Ca^{2+}-dependent gating of $Ca_V1.3$ L-type calcium channels by alternative splicing of a C-terminal regulatory domain, J. Biol. Chem. 283 (2008) 20733–20744.
[84] S.C. Lenhard, R. Strittmatter, W.J. Price, S. Chandra, R.F. White, F.C. Barone, Brain MRI and neurological deficit measurements in focal stroke: rapid throughput validated with isradipine, Pharmacology 81 (2008) 1–10.
[85] C.A. Campbell, K.B. Mackay, S. Patel, P.D. King, J.L. Stretton, S.J. Hadingham, T.C. Hamilton, Effects of isradipine, an L-type calcium channel blocker on permanent and transient focal cerebral ischemia in spontaneously hypertensive rats, Exp. Neurol. 148 (1997) 45–50.

[86] A.L. Coon, D.R. Wallace, C.F. Mactutus, R.M. Booze, L-type calcium channels in the hippocampus and cerebellum of Alzheimer's disease brain tissue, Neurobiol. Aging 20 (1999) 597–603.
[87] M. Ikeda, D. Dewar, J. McCulloch, A correlative study of calcium channel antagonist binding and local neuropathological features in the hippocampus in Alzheimer's disease, Brain Res. 589 (1992) 313–319.
[88] K. Barhwal, S.K. Hota, I. Baitharu, D. Prasad, S.B. Singh, G. Ilavazhagan, Isradipine antagonizes hypobaric hypoxia induced CA1 damage and memory impairment: complementary roles of L-type calcium channel and NMDA receptors, Neurobiol. Dis. 34 (2009) 230–244.
[89] D.A. Sica, Calcium channel blocker class heterogeneity: select aspects of pharmacokinetics and pharmacodynamics, J. Clin. Hypertens. (Greenwich) 7 (2005) 21–26.
[90] H.R. Christensen, K. Antonsen, K. Simonsen, A. Lindekaer, J. Bonde, H.R. Angelo, J.P. Kampmann, Bioavailability and pharmacokinetics of isradipine after oral and intravenous administration: half-life shorter than expected? Pharmacol. Toxicol. 86 (2000) 178–182.
[91] B.L. Sopher, K. Fukuchi, T.J. Kavanagh, C.E. Furlong, G.M. Martin, Neurodegenerative mechanisms in Alzheimer disease. A role for oxidative damage in amyloid beta protein precursor-mediated cell death, Mol. Chem. Neuropathol. 29 (1996) 153–168.
[92] B.L. Sopher, K. Fukuchi, A.C. Smith, K.A. Leppig, C.E. Furlong, G.M. Martin, Cytotoxicity mediated by conditional expression of a carboxyl-terminal derivative of the beta-amyloid precursor protein, Brain Res. Mol. Brain Res. 26 (1994) 207–217.
[93] P.F. Copenhaver, T.S. Anekonda, D. Musashe, K.M. Robinson, T.L. Swanson, T.L. Wadsworth, D. Kretzschmar, R.L. Woltjer, J.F. Quinn, A translational continuum of model systems for evaluating treatment strategies in Alzheimer's disease: isradipine as a candidate drug, Dis. Mod. Mech. 4 (2011) 634–648.
[94] S. Oddo, A. Caccamo, M. Kitazawa, B.P. Tseng, F.M. LaFerla, Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease, Neurobiol. Aging 24 (2003) 1063–1070.
[95] S. Oddo, A. Caccamo, J.D. Shepherd, M.P. Murphy, T.E. Golde, R. Kayed, R. Metherate, M.P. Mattson, Y. Akbari, F.M. LaFerla, Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction, Neuron 39 (2003) 409–421.
[96] M.J. Sinnegger-Brauns, I.G. Huber, A. Koschak, C. Wild, G.J. Obermair, U. Einzinger, J.C. Hoda, S.B. Sartori, J. Striessnig, Expression and 1,4-dihydropyridine-binding properties of brain L-type calcium channel isoforms, Mol. Pharmacol. 75 (2009) 407–414.
[97] L. Lacinova, N. Klugbauer, F. Hofmann, State- and isoform-dependent interaction of isradipine with the alpha1C L-type calcium channel, Pflugers Arch. 440 (2000) 50–60.
[98] J.C. Diaz, O. Simakova, K.A. Jacobson, N. Arispe, H.B. Pollard, Small molecule blockers of the Alzheimer Abeta calcium channel potently protect neurons from Abeta cytotoxicity, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 3348–3353.
[99] S.J. Cherra III, R.K. Dagda, C.T. Chu, Review: autophagy and neurodegeneration: survival at a cost? Neuropathol. Appl. Neurobiol. 36 (2010) 125–132.
[100] S.J. Cherra III, S.M. Kulich, G. Uechi, M. Balasubramani, J. Mountzouris, B.W. Day, C.T. Chu, Regulation of the autophagy protein LC3 by phosphorylation, J. Cell. Biol. 190 (2010) 533–539.
[101] J. Striessnig, H.J. Bolz, A. Koschak, Channelopathies in $Ca_v1.1$, $Ca_v1.3$, and $Ca_v1.4$ voltage-gated L-type Ca^{2+} channels, Pflugers Arch. 460 (2010) 361–374.
[102] G.D. Clifton, R.A. Blouin, C. Dilea, H.F. Schran, A.E. Hassell, L.M. Gonasun, T.S. Foster, The pharmacokinetics of oral isradipine in normal volunteers, J. Clin. Pharmacol. 28 (1988) 36–42.
[103] A.P. Sen, P. Boksa, R. Quirion, Brain calcium channel related dihydropyridine and phenylalkylamine binding sites in Alzheimer's, Parkinson's and Huntington's diseases, Brain Res. 611 (1993) 216–221.
[104] J.H. Park, Y.S. Park, S.Y. Rhim, O.H. Jhee, S.H. Kim, S.C. Yang, M.H. Lee, L.M. Shaw, J.S. Kang, Quantification of isradipine in human plasma using LC–MS/MS for pharmacokinetic and bioequivalence study, J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci. 877 (2009) 59–64.
[105] R. Gaggi, R. Dall'Olio, P. Roncada, A.M. Gianni, Peculiar effects of isradipine and darodipine on the rat brain dopaminergic system, Gen. Pharmacol. 26 (1995) 303–308.
[106] L.M. Sanftner, J.A. Gibbons, M.I. Gross, B.M. Suzuki, F.C. Gaeta, K.W. Johnson, Cross-species comparisons of the pharmacokinetics of ibudilast, Xenobiotica 39 (2009) 964–977.