PERSPECTIVE

Tetrahydrohyperforin: a neuroprotective modified natural compound against Alzheimer's disease

According to the World Health Organization (WHO), a total of 35.6 million cases of dementia were estimated in 2010, with close to 7.7 million new cases each year. In 2008, WHO declared dementia a priority condition. 90% of all dementia cases are considered to be Alzheimer's disease (AD). Therefore, great effort was made to understand the etiology of the disease and stop or slow down AD progression.

Several different therapies have been developed, including active and passive immunotherapy against the amyloid-β (Aβ) protein, drugs targeting cholinergic synapses (*i.e.*, donezepil and rivastigimine) and glutamatergic synapses (*i.e.*, memantine), and microtubule stabilization by targeting tau protein, among many others. Unfortunately, these drugs do not directly attack the disease, but rather act by compensating the synaptic transmission loss, and therefore, show only short-term beneficial effects.

For over ten years we have studied the effects of tetrahydrohyperforin, a semisynthetic derivative of the natural compound hyperforin found in St. John's wort (*Hypericum perforatum*). Hyperforin shows low stability and bioavailability. The chemically reduced derivative tetrahydrohyperforin is more stable and therefore more efficient in treatments. Injecting tetrahydrohyperforin into wild-type and double transgenic mice that model AD ($APP_{swe}/PS1^{\Delta E9}$), we have found positive effects at a molecular, cellular and cognitive level. Among the effects of tetrahydrohyperforin, we found that it prevents deposition of Aβ peptides, prevents abnormal tau phosphorylation, and mitigates synaptotoxicity. It also increases adult neurogenesis in wild-type and $APP_{swe}/PS1^{\Delta E9}$ mice. These molecular and cellular changes are most likely related to tetrahydrohyperforin's ability to prevent cognitive deficits when administered to young or old $APP_{swe}/PS1^{\Delta E9}$ mice.

In the present perspective, we are going to present and compare the effects of tetrahydrohyperforin and hyperforin, propose a mechanism of action based on recently published data, and finally, will discuss future possibilities of tetrahydrohyperforin in AD therapy.

Tetrahydrohyperforin is a derivative of the natural compound hyperforin: St. John's wort has been used since ancient times for its antidepressive, antiinflammatory and antiseptic properties as part of popular medicine traditions. The main active compound of this plant is hyperforin, a bicyclic polyprenylated acylphloroglucinol. Once extracted, the compound is highly unstable when exposed to light, heat, or air. More stable analogues of hyperforin were sought by systematic chemical modification and resulted in the semisynthetic compound tetrahydrohyperforin, where two carbonyl groups were reduced to hydroxyl groups. Tetrahydrohyperforin shows greater chemical stability and oral bioavailability.

Most of the pharmacological effects described for hyperforin have been described for tetrahydrohyperforin, as well. In particular, among the shared effects described for the central nervous system (CNS), there are: enhanced performance in memory paradigm tests, reduction in Aβ plaque size, and decrease of astrogliosis and various inflammation markers in brain.

Even more interesting are the discoveries of the neuroprotective properties of tetrahydrohyperforin. Next, we are going to describe the molecular effects of tetrahydrohyperforin in a mouse model of AD and how these effects prevent memory loss.

Tetrahydrohyperforin generates neuroprotective effects and prevents memory loss in double transgenic mice modeling AD: In our laboratory, we established that hyperforin is able to disaggregate Aβ peptides *in vitro* and have shown that it prevents Aβ neurotoxicity in rat hippocampus *in vivo* (Dinamarca et al., 2006). We have also reported that intraperitoneal administration of this drug in the most commonly used mouse model of AD ($APP_{swe}/PS1^{\Delta E9}$) occludes cognitive deficit, reducing the Aβ plaque size and the oxidative damage in cortex and hippocampus (Cerpa et al., 2010). Once the effects of hyperforin were clear, we aimed at studying tetrahydrohyperforin to elucidate whether it had similar effects.

Experiments showed that in $APP_{swe}/PS1^{\Delta E9}$ mice, tetrahydrohyperforin promotes non-amyloidogenic amyloid precursor protein (APP) processing by inhibiting γ-secretase-mediated cleavage of APP's C-terminal fragment, C99 (Inestrosa et al., 2011). As mentioned before, it also increases neurogenesis in the dentate gyrus of wild-type and $APP_{swe}/PS1^{\Delta E9}$ mice (Abbott et al., 2013). It was further established that tetrahydrohyperforin prevents mitochondrial Ca^{2+} overload, and therefore protects against mitochondrial dysfunction (Zolezzi et al., 2013). Overall, tetrahydrohyperforin both *in vitro* and *in vivo* has synapto- and neuroprotective effects against Aβ oligomers.

Although the data obtained was relevant, one important issue was not solved: the mechanism of action through which tetrahydrohyperforin could be generating these effects. Several hypotheses have been proposed during the years. One of them takes into consideration the fact that hyperforin can act as an N-methyl D-aspartate (NMDA) receptor antagonist, and therefore, could block reactive oxygen species (ROS) formation (Kumar et al., 2006). Unfortunately, there is no further advance in this work. Another possibility is that tetrahydrohyperforin, similar to hyperforin, could activate the transient receptor potential cation channel subfamily 6 (TRPC6) (Leuner et al., 2007). TRPC channels are non-selective cationic channels thought to be composed of six membrane spanning segments with intracellular amino- and carboxy-termini. Based on sequence similarity these were divided into two subfamilies: TRPC1/4/5 and TRPC3/6/7. TRPC6 channels are principally Ca^{2+} permeable channels ubiquitously found in the body, but importantly located in the postsynaptic density of hippocampal neurons.

Within the CNS, TRPC3/6 channels have key roles in neuronal survival by transmitting brain-derived neurotrophic factor (BDNF) signaling and promoting gene expression *via* the cAMP response element-binding protein (CREB). TRPC3 channels are involved in hippocampal excitability. TRPC7 channels are found only in embryonic stages, so they

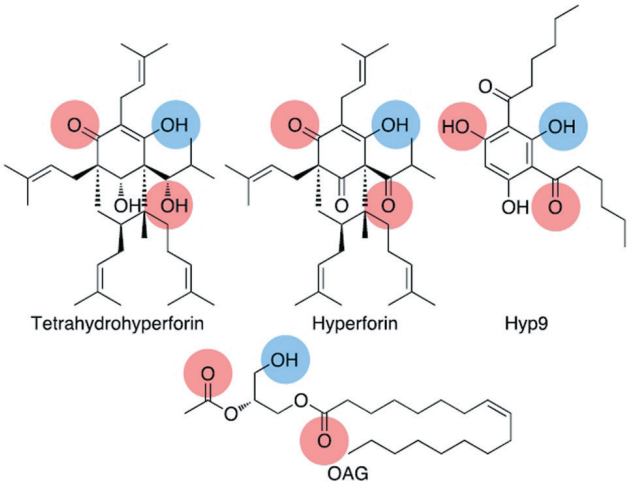

Figure 1 Known transient receptor potential cation channel subfamily 6 (TRPC6) activators share a common potential pharmacophore.
Conformers of each compound were generated by short runs of molecular dynamics simulation and were subsequently aligned to maximize structural and pharmacophoric overlay. The three-dimensional pharmacophore alignment was plotted in two dimensions for clarity. Red: Aligned hydrogen bond acceptors; blue: aligned hydrogen bond donors.

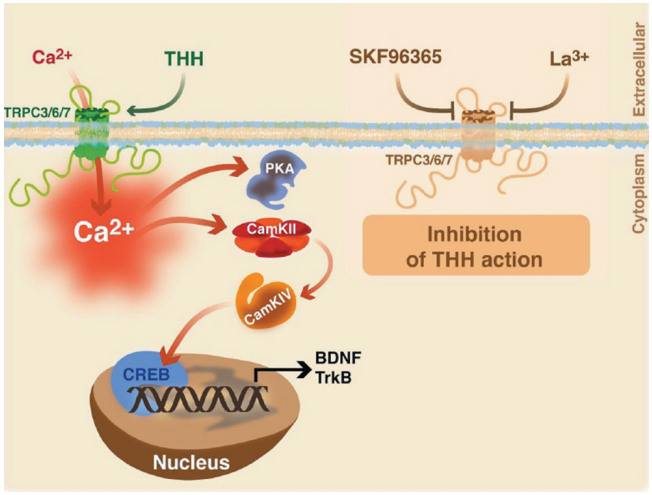

Figure 2 Proposed mechanism for tetrahydrohyperforin (THH) action.
Tetrahydrohyperforin activates transcient receptor potential canonical channels, subfamily 3/6/7 (TRPC3/6/7) channels, triggering the opening of the channels. Consequently there is an increase in intracellular calcium levels, which activates different kinases like PKA and calcium calmodulin kinase II (CaMKII). CaMKII activation leads to CaMKIV activation which generates the transcription of the cAMP response element factor (CREB) promoter regulated genes, brain-derived neurotrophic factor (BDNF) and tyrosine receptor kinase B (TrkB). When tetrahydrohyperforin is co-incubated with lanthanum (La^{3+}) or SKF96365, blockers of TRPC and TRPC3/6/7 channels, respectively, the action of tetrahydrohyperforin is inhibited.

should not be relevant in the mechanism of adult neuroprotection mediated by tetrahydrohyperforin.

Next, we are going to focus our attention on the recent discoveries regarding tetrahydrohyperforin and TRPC6 channels.

Tetrahydrohyperforin's mechanism of action and working model: Since it was already reported that hyperforin could specifically activate TRPC6, our working hypothesis was straightforward: To test whether tetrahydrohyperforin could activate TRPC6 channels and through this, generate Aβ synapto- and neuroprotection.

In order to test this hypothesis we performed electrophysiological, biochemical, behavioral and *in silico* studies. With help of non-specific and specific TRPC3/6/7 channel blockers (lanthanum and SKF96365, respectively) we tested whether previously reported beneficial effects of tetrahydrohyperforin were dependent on TRPC channel activation, more specifically the TRPC3/6/7 subfamily of TRPC channels.

Electrophysiological experiments demonstrated that, when Aβ oligomers were added to the recording solution, hippocampal slices showed a decrease in the field excitatory postsynaptic potential (fEPSP) in basal activity and after long term potentiation induction. But when tetrahydrohyperforin was co-administered with Aβ oligomers, the effect was significantly recovered (Montecinos-Oliva et al., 2014). Interestingly, when co-administering the TRPC channel blockers lanthanum or SKF96365 with a solution of Aβ oligomers plus tetrahydrohyperforin, the protective effect of tetrahydrohyperforin was lost. These experiments show that by blocking TRPC3/6/7 channels, tetrahydrohyperforin is not able to exert the positive effects on the fEPSP amplitude. We discarded any structural changes in the hippocampus, since there was no apparent variation in protein levels of expression after incubation with tetrahydrohyperforin and/or SKF96365.

Next, we treated wild-type mice with 6 mg/kg of tetrahydrohyperforin through i.p. injections, three times a week for 10 weeks. We had previously established that this treatment causes mice to have a better performance in memory tests, *i.e.*, low escape latencies in the Morris water maze (MWM). Particularly interesting was the fact that administration of the TRPC3/6/7 channel blocker SKF96365 caused higher escape latencies in the group of mice treated with tetrahydrohyperforin (Montecinos-Oliva et al., 2014). The inhibition of TRPC3/6/7 channels prevented the positive effects seen in animals treated only with tetrahydrohyperforin. This indicates that the activation of TRPC3/6/7 channels is a requirement for tetrahydrohyperforin action.

Both hyperforin and its semisynthetic derivative tetrahydrohyperforin possess an acylphloroglucinol scaffold. Hyperforin was shown to selectively activate TRCP6 channels while TRPC1/3/4/5 channels remained unaffected (Leuner et al., 2007). Starting from hyperforin, a series of diacylated phloroglucinol compounds was synthesized of which several (Hyp1, Hyp5, Hyp7, Hyp8, Hyp9) were found to stimulate TRPC6 channels without effecting TRPC3/7 channels (Leuner et al., 2010). Further, endogenous diacylglycerols (DAG), *e.g.*, 1-oleoyl-2-acetyl-sn-glycerol (OAG), are thought to directly activate the TRPC3/6/7 subgroup while no effect on TRPC1/4/5 was observed. Extending earlier works of Leuner et al. (2010), we have proposed by *in silico* conformational analysis that tetrahydrohyperforin, hyperforin, Hyp9, and OAG share a potential pharmacophore of two hydrogen bond acceptors, one hydrogen bond donor, and an extended lipophilic contact surface (**Figure 1**) (Montecinos-Oliva et al., 2014). This result indicates that these TRPC activators are potentially able to interact with their target channel on a similar molecular basis.

Our research has led us to propose that tetrahydrohyperforin is an agonist of the TRPC3/6/7 subfamily of channels. The compound probably causes an increase in intracellular

calcium concentrations and generates stronger synaptic responses, which might be compensating the effects of Aβ oligomers. The effect on synaptic response was described before as an increase in synaptic protein levels of APP-PS1 animals, treated with tetrahydrohyperforin. Also, there is a quick increase in synaptic activity just minutes after addition of tetrahydrohyperforin in the bathing solution of hippocampal slices. This could be associated to the rapid calcium entry, after TRPC3/6/7 channel activation. Activation of TRPC6 channels has also been linked to calcium calmodulin kinase IV and cAMP response element-binding (CaMKIV/CREB) signaling in hippocampal neurons (Tai et al., 2008) (**Figure 2**). Activation of the CREB transcription factor promotes the expression of the neurotrophin receptor TrkB, which binds BDNF. It is even more relevant since the same activation of CREB leads to BDNF expression. Therefore, tetrahydrohyperforin could be generating an increase in cell survival by increasing the expression of the receptor and the ligand involved in the process. CREB signaling is related to hippocampal neurogenesis, particularly in the dentate gyrus, an adult neurogenesis niche. This and the fact that TRPC6 channels are mainly found in the dentate gyrus within the hippocampus, further support the relationship between TRPC6 channels and tetrahydrohyperforin. Interestingly, failures in calcium calmodulin kinase IV (CaMKIV) are related to APP-induced neuronal death. Further analysis is required in order to determine which specific channel, of the TRPC3/6/7 subfamily, is activated by tetrahydrohyperforin.

Future guidelines: Deciphering the mechanism of action of tetrahydrohyperforin is a fundamental step in order to establish the suitability of this compound as a potential agent for AD chemotherapy. Our results show that TRPC3/6/7 activation by tetrahydrohyperforin is based on the electrophysiological and behavioral responses described for this compound. Among the members of the TRPC3/6/7 subfamily, the most likely candidate to be targeted by tetrahydrohyperforin is TRPC6. However, the lack of an atomic resolution structure of any TRPC channel and the limited availability of structure-activity relationship (SAR) data of TRPC small molecule modulators, currently hamper rational drug design for these channels. At the same time, once a specific blocker of TRPC6 becomes available, we could directly study the relevance of TRPC6 activation on the action mechanism of tetrahydrohyperforin.

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