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## Cellular neurobiology of hyperforin

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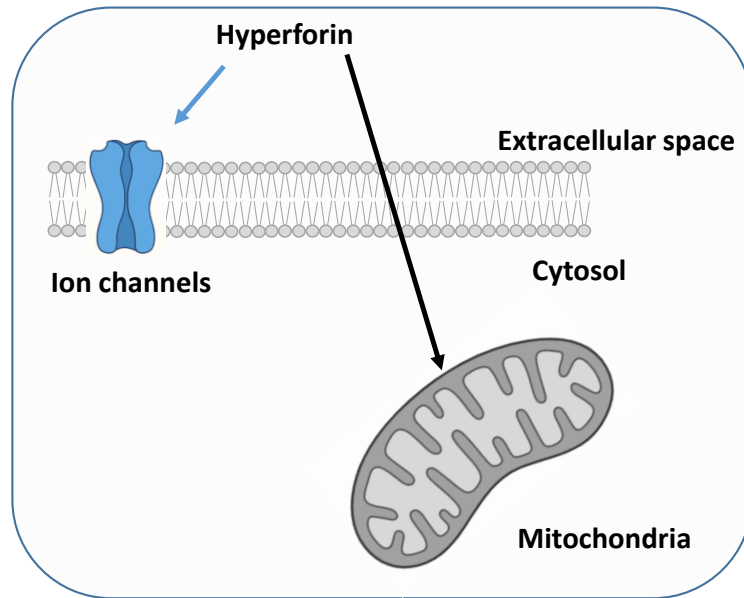
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**Keywords:** brain; hyperforin; antidepressant; ion channels; neuronal cell death; neuroprotection; mitochondria.



**Graphical abstract**

## **Abstract**

Hyperforin is a phloroglucinol derivative isolated from the medicinal plant *Hypericum perforatum* (St John's wort, SJW). This lipophilic biomolecule displays anti-bacterial, pro-apoptotic, anti-proliferative and anti-inflammatory activities. In addition, *in vitro* and *in vivo* data showed that hyperforin is a promising molecule with potential applications in neurology and psychiatry. For instance, hyperforin possesses antidepressant properties, impairs the uptake of neurotransmitters, and stimulates the BDNF/TrkB neurotrophic signalling pathway, the adult hippocampal neurogenesis, and the brain homeostasis of zinc. In fact, hyperforin is a multi-target biomolecule with a complex neuropharmacological profile. However, one prominent pharmacological feature of hyperforin is its ability to influence the homeostasis of cations such as  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Zn}^{2+}$  and  $\text{H}^+$ . So far, the pathophysiological relevance of these actions is currently unknown. The main objective of the present work is to provide an overview of the cellular neurobiology of hyperforin, with a special focus on its effects on neuronal membranes and the movement of cations.

**Keywords:** brain; hyperforin; antidepressant; ion channels; neuronal cell death; neuroprotection; mitochondria.

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## Introduction

The plant St John's wort (SJW) (*Hypericum perforatum*) has been used for centuries as a folk medicine for the treatment of various conditions (Bilia, Gallori, & Vincieri, 2002) (Wolfe, Seelinger, & Schempp, 2014). Neuropharmacology is an important field of application of SJW extracts due to their ability to alleviate depressive symptoms (Medina, Martinez-Poveda, Amores-Sanchez, & Quesada, 2006). Their efficiency seems comparable to the one of synthetic antidepressants like selective serotonin reuptake inhibitors (SSRI) but with fewer side effects (Apaydin et al., 2016; Linde et al., 1996; Ng, Venkatanarayanan, & Ho, 2017). This finding paved the way for *in vivo* and *in vitro* studies aiming at providing a molecular understanding of the antidepressant properties of SJW extracts. Several pharmacologically active molecules were identified such as hypericin, adhyperforin and hyperforin. Hypericin was originally described as the main antidepressant molecule but subsequent studies questioned this view and provided evidence for a central role of hyperforin (Beerhues, 2006; Cervo et al., 2002; S. S. Chatterjee, Bhattacharya, Wonnemann, Singer, & Muller, 1998; Zanolini, 2004). It is important to mention that, in addition to hyperforin, several bioactive molecules contribute to the clinical efficacy of the extracts (Butterweck & Schmidt, 2007).

Hyperforin is a phloroglucinol derivative present in high amounts in leaves and flowers of SJW (Beerhues, 2006) (Herraiz & Guillen, 2018). It exhibits various pharmacological properties of medical interest with antibacterial (Gurevitch et al, 1971), anti-inflammatory or anti-tumor activities (Medina et al., 2006; Pia Schiavone et al., 2014) (Menegazzi, Masiello, & Novelli, 2020). Hyperforin has also been described as a larvicidal agent due to its toxicity against the larvae of the mosquito *Culex pipiens* (Mitsopoulou, Vidali, Koliopoulos, Couladouros, & Michaelakis, 2014). More recently, hyperforin was shown to activate thermogenesis *in vitro* and *in vivo*, and introduced as an attractive anti-obesity molecule (Chen et al., 2021). However, one major disadvantage of hyperforin is its instability, particularly when exposed to light and air (Orth, Rentel, & Schmidt, 1999). This has stimulated the development of more stable analogues such as aristoforin, tetrahydrohyperforin, or 2,4-diacylphloroglucinol compounds.

So far, *in vitro* and *in vivo* data suggest that hyperforin may constitute a promising molecule with potential applications in neurology and psychiatry. However, its neuropharmacological profile is far from being fully clarified. As a lipophilic compound (Spiess et al., 2023), hyperforin readily crosses biological membranes and penetrates the brain (Eckert et al., 2004). It can insert into membranes and facilitates the activation of the cytosolic phospholipase A2 (Hoffmann et al., 2010). *In vitro*, hyperforin influences the flexibility and fluidity of crude brain membranes (Eckert & Muller, 2001) (Eckert et al., 2004) (Keksel et al., 2019). *In vivo*, oral administration of hyperforin to mice also changes the fluidity of brain membranes (Eckert et al., 2004). ~~The overall effects of hyperforin on the fluidity of biological membranes seems difficult to predict due to the multifaceted properties of this~~

bioactive molecule. (Eckert & Muller, 2001) studied the effects of hyperforin on different fluidity parameters of brain membranes. They conducted fluidity measurements using three distinct fluorescent probes and could show that hyperforin decreased the flexibility of acyl chains in the membrane hydrocarbon core whereas it also exerted a fluidizing effect in the hydrophilic membrane region of the phospholipid heads. Moreover, low concentrations of hyperforin (e.g. 0.3  $\mu\text{M}$ ) decreased the membrane fluidity without altering the membrane bulk fluidity of the neuronal membranes. Altogether, these data suggest that the pharmacological properties of hyperforin could be attributed, at least in part, to its physicochemical interaction with membranes. It is plausible that multiple cellular targets and neurochemical changes contribute to the antidepressant action of hyperforin. Depressive disorders are complex psychiatric diseases with numerous interrelated signalling pathways and neurotransmitter systems (Dean & Keshavan, 2017) (Fries et al, 2023). Therefore, a complete understanding of the neurobiology of depression and its treatment remains a daunting task.

In addition to its actions on phospholipid membranes, hyperforin alters the functioning of membrane-embedded proteins such as ion channels (see below for further details). Experiments conducted on rats and humans indicate that the *in vivo* plasma levels of hyperforin is  $<0.5 \mu\text{M}$  (Biber, Fischer, Romer, & Chatterjee, 1998) (Schulz, Schurer, Bassler, & Weiser, 2005). These pharmacokinetics data are important to determine the brain targets of hyperforin. The overall goal of this work is to provide an overview of the cellular neurobiology of hyperforin, with a special focus on its effects on neuronal membranes and the cellular homeostasis of ions (Figure 1).

### **1. Hyperforin and membrane proteins**

Hyperforin has been reported to inhibit the uptake of neurotransmitters like serotonin, norepinephrine, dopamine, GABA, and glutamate with  $\text{IC}_{50}$ s of  $\approx 80\text{-}200 \text{ nM}$  (S. S. Chatterjee, Bhattacharya, et al., 1998) (Singer, Wonnemann, & Muller, 1999) (Wonnemann, Singer, & Muller, 2000) (Marsh & Davies, 2002). Hyperforin was also shown to favour the release of neurotransmitters (e.g. glutamate, aspartate, GABA) from synaptosomes (Chatterjee et al, 2001). This led to the hypothesis that this bioactive molecule may act by promoting an unspecific neurotransmitter release instead of inhibiting the re-uptake of neurotransmitters (Griffith, Varela-Nallar, Dinamarca, & Inestrosa, 2010). Of note, hyperforin does not inhibit the activity of the human monoamine oxidase A (MAO-A) (Herraiz & Guillen, 2018). Hyperforin exerts a dual action on the organic anion-transporting polypeptide OATP2B1 that is highly expressed in the brain. OATP2B1 is thought to play important roles in drug absorption and elimination. On the one hand, hyperforin inhibits the OATP2B1-mediated transport, which may impair intestinal absorption of drugs. On the other hand, OATP2B1 favours the cellular accumulation of hyperforin (Schafer, Bock, & Meyer Zu Schwabedissen, 2018) (Schafer, Potterat, Seibert, Fertig, & Meyer Zu Schwabedissen, 2019). This illustrates the complexity of the cellular actions of hyperforin, notably when considering its interaction with drugs. It also inhibits the high-affinity choline uptake in rat brain synaptosomes with an  $\text{IC}_{50}$  of  $8.5 \mu\text{M}$  (Buchholzer, Dvorak,

Chatterjee, & Klein, 2002). Choline is involved in the synthesis of acetylcholine. The brain cholinergic system is an important neuromodulatory system regulating sensory and motor functions. Its alteration is likely to influence various brain processing such as cognition, emotions, and/or locomotion.

One prominent feature of hyperforin is its ability to alter the transmembrane transport of ions. In most instances, it exerts an inhibitory action on multiple neuronal ion channels. In rat hippocampal neurons, 1  $\mu\text{M}$  hyperforin inhibits partially and reversibly all the voltage-gated ion currents tested ( $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$ ) as well as AMPA- and GABA-activated currents (S. Chatterjee et al., 1999). In isolated cerebellar Purkinje neurons, concentrations of 0.4-0.8  $\mu\text{M}$  hyperforin shift the I/V curves and reversibly depress the amplitude of voltage-gated  $\text{Ca}^{2+}$  currents of P-type (Fisunov et al., 2000). Contrasting results have been obtained concerning the effects of hyperforin on NMDA receptors.  $\text{Ca}^{2+}$  imaging experiments showed that hyperforin (10  $\mu\text{M}$ ) inhibits NMDA-induced  $\text{Ca}^{2+}$  entry in cortical neurons in primary cultures (Kumar et al., 2006). In freshly isolated hippocampal neurons, hyperforin inhibits NMDA-activated currents with an  $\text{IC}_{50}$  of  $\approx 3 \mu\text{M}$  (S. Chatterjee et al., 1999). However, a concentration of 1  $\mu\text{M}$  hyperforin does not affect currents generated in response to NMDA receptor activation in hippocampal pyramidal neurons from brain slices (Pochwat et al., 2018). Hyperforin, which is not a sodium ( $\text{Na}^+$ ) ionophore by itself elevates the free intracellular  $\text{Na}^+$  concentration (Singer et al., 1999). Contrasting results have been obtained regarding the contribution of the  $\text{Na}^+/\text{H}^+$  exchanger. Hyperforin was thought to stimulate the activity of the  $\text{Na}^+/\text{H}^+$  exchanger in brain synaptosomal preparations (Wonnemann et al., 2000) but no such stimulatory effect was found in human platelets (Treiber, Singer, Henke, & Muller, 2005). Hence, the activity of TRPC6, a type of cationic channels carrying  $\text{Ca}^{2+}$  and  $\text{Na}^+$  ions, is enhanced by hyperforin (see below) and may also contribute to these hyperforin-triggered intracellular  $\text{Na}^+$  rises. Of note, the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (Treiber et al., 2005) as well as the  $\text{Na}^+/\text{K}^+$  ATPase (Wonnemann et al., 2000) are unaffected by hyperforin.

As briefly introduced above, TRPC6 channels have been identified as molecular targets of hyperforin. Original work conducted on dissociated hippocampal neurons showed that, acutely applied, hyperforin evokes an inward depolarizing current that is mainly carried by  $\text{Ca}^{2+}$  and  $\text{Na}^+$  ions with an  $\text{EC}_{50}$  of  $\sim 9 \mu\text{M}$  (S. Chatterjee et al., 1999). This hyperforin-induced current was found to have a pharmacological profile reminiscent of transient receptor potential canonical (TRPC) channels (Treiber et al., 2005), a group of nonselective  $\text{Ca}^{2+}$ -permeable cation channels comprising seven members (TRPC1-TRPC7) (Abramowitz & Birnbaumer, 2009) (Nilius & Szallasi, 2014). Subsequent studies showed that TRPC6 channels, and no other TRPC member, are recruited by hyperforin (Leuner et al., 2007).  $\text{Ca}^{2+}$  imaging experiments confirmed that, acutely applied, hyperforin evokes  $\text{Ca}^{2+}$  rises in smooth muscle cells (Koch and Chatterjee, 2001), mouse cortical neurons in slices (Marsh and Davies, 2002) and culture (Tu, Gibon, & Bouron, 2010), in rat cortical synaptosomes (Chatterjee et al., 2001), and human



platelets (Treiber et al., 2005). Since then, hyperforin (or its analog Hyp9 (Zhou et al., 2018)) has been shown to elicit a TRPC6-dependent cytosolic  $\text{Ca}^{2+}$  rise in multiple cell types such as aortic vascular smooth muscle cells (Y. Ding et al., 2011), podocytes (F. Ding et al., 2014), neural precursor cells (Griesi-Oliveira et al., 2015), glomerular mesangial cells (Soni & Adebisi, 2016), HEK-293 cells (H. Zhang et al., 2016) or 16HBE cells. Altogether, these experimental data identify TRPC6 channels as molecular targets of hyperforin. Several groups took advantage of this pharmacological property to investigate the properties and functions of TRPC6. For instance, hyperforin enhances in a TRPC6-dependent manner stretch-activated  $\text{Ca}^{2+}$  responses (Takada, Furuya, & Sokabe, 2014), the phosphorylation of transcription factors like CREB (Jia, Zhou, Tai, & Wang, 2007) (Cardile et al., 2023) (Gibon et al., 2013) and AP-1 (Thiel & Rossler, 2017) (Scheuble, Rossler, Ulrich, & Thiel, 2020). The group of Menegazzi recently confirmed this latter finding by showing that hyperforin induces a transient activation of AP-1 in melanoma cells (Cardile et al., 2023).

It also stimulates neuronal axonal sprouting (Leuner et al., 2007), keratinocytes differentiation (Muller et al., 2008), modulates dendritic spine morphology (Leuner et al., 2013) and the firing properties of hippocampal neurons (El Hamdaoui et al., 2022) in a TRPC6-dependent manner.

However, questions have been raised about the molecular identity of the hyperforin-activated cation channels. Indeed, Sell et al showed that hyperforin is a protonophore that activates a proton ( $\text{H}^+$ ) conductance of the plasma membrane unrelated to TRPC6 (Sell, Belkacemi, Flockerzi, & Beck, 2014). The protonophore properties of hyperforin were in fact originally introduced by (Roz & Rehavi, 2003) who demonstrated that hyperforin collapses the pH gradient across synaptic vesicle membranes.

Mitochondria are thus regarded as intracellular targets of hyperforin. For instance, low concentrations of hyperforin ( $<0.5\mu\text{M}$ ) increase the mitochondrial membrane potential ( $\Delta\psi\text{m}$ ) and ATP production in the oligodendrocyte-type 2 astrocyte progenitor cell line CG-4 (Y. Wang et al., 2011). Depolarizing effects of hyperforin on  $\Delta\psi\text{m}$  have been repeatedly reported in a wide diversity of cell types. For instance, hyperforin and its derivative dicyclohexylammonium salt of hyperforin (DCHA-HF) induce a loss of  $\Delta\psi\text{m}$  in MT-450 cells (Schempp et al., 2002), lymphatic endothelial cells (Rothley et al., 2009), cortical neurons (Tu et al., 2010), K562 cells (J. Y. Liu et al., 2011), hepatocellular carcinoma cell line (Chiang et al., 2017), human leukemic HL-60 cells (Wiechmann, Muller, Fischer, Jauch, & Werz, 2015), acute myeloid leukaemia cells U937 (Merhi et al., 2011), and the glioblastoma cell lines U-87 MG and GBM8401 (Hsu, Chen, Wu, & Chung, 2020). In fact, hyperforin seems to interfere directly with mitochondrial membranes (Wiechmann et al., 2015), increases the proton conductance (Sell et al., 2014) (Roz & Rehavi, 2003), induces a loss of  $\Delta\psi\text{m}$  and promotes the mitochondrial release of cations like  $\text{Ca}^{2+}$  and zinc ( $\text{Zn}^{2+}$ ) (Tu et al., 2010) (Hsu et al., 2020). A recent work further established mitochondria as direct targets of hyperforin (Chen et al., 2021). The authors showed that hyperforin alters the respiration and upregulates several mitochondrial electron transport chain

proteins like *Atp5a*, *Uqcrc2*, *Mtco1*, *Sdhb*, and *Ndufb8* in C3H10T1/2-derived adipocytes (Chen et al., 2021). It depresses the mitochondrial activity of human hepatocytes (Komoroski et al., 2004).

Moreover, hyperforin affects cellular respiration and increases the number of mitochondria in human mesenchymal stem cell-derived adipocytes and in primary inguinal adipocytes. Furthermore, (Chen et al., 2021) identified dihydrolipoamide S-acetyltransferase (*Dlat*) (a component of the pyruvate dehydrogenase complex) as a molecular target of hyperforin. As discussed above, the involvement of TRPC6 channels to the cellular actions of hyperforin has been a controversial point. However, the group of K. Friedland has recently provided compelling evidence for a direct action of hyperforin on TRPC6 channels by identifying a hyperforin-binding motif located in the C-terminus region of the channel (<sup>777</sup>LLKL<sup>780</sup>) (El Hamdaoui et al., 2022).

Is hyperforin a protonophore (Sell et al., 2014) or a modulator of TRPC6 channel activity (Leuner et al., 2007) ? These two properties may not be mutually exclusive. On the one hand, mitochondria are well established intracellular targets of hyperforin (J. Y. Liu et al., 2011; Rothley et al., 2009; Schempp et al., 2002; Tu et al., 2010; Wiechmann et al., 2015). On the other hand, multiple and independent studies provided evidence for a contribution of TRPC6 to the cellular actions of hyperforin (Griesi-Oliveira et al., 2015) (Y. Ding et al., 2011) (Takada et al., 2014) (Thiel & Rossler, 2017) (El Hamdaoui et al., 2022). A connection between TRPC6 and mitochondria has been highlighted (Ko & Kang, 2017). Indeed, the presence TRPC channels in mitochondria has been documented but exclusively TRPC3 (Feng et al., 2013). So far, no other TRPC members, including TRPC6, has been identified in mitochondrial membranes. Like any protonophore hyperforin causes an acidification of the cytosol (Koch & Chatterjee, 2001) (Froestl, Steiner, & Muller, 2003). However, TRPC6 channels activity is inhibited by acidic pH (Berna-Erro et al., 2014) and the hyperforin-induced acidosis should inhibit (not promote) Ca<sup>2+</sup> entry through TRPC6. Thus, the hyperforin-induced TRPC6-dependent Ca<sup>2+</sup> entry is not caused by a pH change but the protonophore properties of hyperforin have to be kept in mind when using this bioactive molecule as a TRPC6 activator.

In addition to ion channels, hyperforin regulates membrane receptors such as  $\beta$ 1-adrenergic receptors ( $\beta$ 1-AR). For instance, a chronic hyperforin treatment causes the internalization of  $\beta$ 1-AR, reduces cAMP levels and  $\beta$ 1-adrenergic responsiveness (Jakobs et al., 2013). Therefore, by influencing G-protein-coupled receptors, hyperforin alters downstream intracellular signalling processes.

## 2. Cytotoxicity of hyperforin

A long term hyperforin treatment can cause the death of neural cells. For instance, a 24h treatment with 10  $\mu$ M induces the apoptosis of ~10% of astrocytes (Hostanska, Reichling, Bommer, Weber, & Saller, 2003). However, a 12h treatment with 1 (Huang et al., 2017) or 10  $\mu$ M hyperforin (Froestl et al., 2003) does not affect the survival of PC12 cells. Contrasting results have been obtained on hippocampal neurons. When applied overnight hyperforin causes cell death at concentrations >300 nM with a very significant toxicity at 1  $\mu$ M (H. Zhang et al., 2016), whereas a 24h treatment with 0.01-1

$\mu\text{M}$  hyperforin has no effect on the viability of hippocampal neurons (Dinamarca, Cerpa, Garrido, Hancke, & Inestrosa, 2006). However, a 10  $\mu\text{M}$  concentration of hyperforin causes nearly 25-30% of neuronal cell loss (Dinamarca et al., 2006). It is thus necessary to assess the impact of hyperforin on neuronal cell survival before conducting long-term applications experiments. It is important to mention that rodents tolerate single or repeated intraperitoneal (or intracranial) injections of hyperforin, indicating this biomolecule is safe *in vivo*.

### 3. Hyperforin and mood disorders

Hyperforin displays antidepressant properties (Beerhues, 2006; Cervo et al., 2002; Medina et al., 2006) (Table 1). Its efficacy has been demonstrated in several animal models of depression (S. S. Chatterjee, Bhattacharya, et al., 1998) (Pochwat et al., 2018) (Szewczyk et al., 2019). It was originally proposed to act like classical synthetic antidepressants by inhibiting the re-uptake of neurotransmitters but this hypothesis has not yet been clearly validated experimentally. Interestingly, hyperforin and its derivative tetrahydrohyperforin have BDNF-like neurotrophic properties (Gibon et al., 2013; Heiser et al., 2013) and stimulate the production of neurons in the hippocampus of adult mice (Abbott, Calderon Toledo, Aranguiz, Inestrosa, & Varela-Nallar, 2013). The BDNF/TrkB neurotrophic signalling pathway and the adult hippocampal neurogenesis could be two key properties responsible of the antidepressant actions of hyperforin (Szewczyk et al., 2019). Recent data showed that TRPC6 channels play a central role in the antidepressant properties of hyperforin. Indeed, TRPC6 deficient mice display a depression- and anxiety-like behavior. A hyperforin treatment abrogates these depressive/anxious phenotypes (El Hamdaoui et al., 2022).

Interestingly, hyperforin influences the homeostasis of  $\text{Zn}^{2+}$ , a cation exerting antidepressant activity and thought to play a role in the pathogenesis of mood disorders (Nowak, Szewczyk, & Pilc, 2005) (Swardfager et al., 2013). Hyperforin releases  $\text{Zn}^{2+}$  from mitochondria (Tu et al., 2010), changes the  $\text{Zn}^{2+}$  storage capacity of the brain by upregulating the expression of the  $\text{Zn}^{2+}$ -binding proteins metallothioneins (Gibon, Richaud, & Bouron, 2011), and increases the concentration of  $\text{Zn}^{2+}$  in the hippocampus (Szewczyk et al., 2019). Further *in vitro* and *in vivo* experimental investigations are needed to better understand the interrelation between hyperforin, brain  $\text{Zn}^{2+}$  homeostasis and depressive disorders.

### 4. Hyperforin and Alzheimer's disease

Hyperforin is a memory-enhancement molecule (Klusa, Germane, Noldner, & Chatterjee, 2001). Its neurobiological properties have been particularly studied in cellular and animal models of Alzheimer's disease (Griffith et al., 2010) (Table 2). Hyperforin (or its derivative tetrahydrohyperforin) exerts beneficial effects *in vivo* and *in vitro* by reducing the formation of  $\beta$ -amyloid oligomers, the hyperphosphorylation of tau and against memory impairments (Griffith et al., 2010; Inestrosa et al., 2011) (Dinamarca et al., 2006). For instance, *in vitro*, hyperforin (0.5  $\mu\text{M}$ ) reduces the intracellular APP

levels of PC12 cells stably expressing human APP by stimulating its secretory processing (Froestl et al., 2003).

Cultured hippocampal neurons prepared from a genetic mouse model of Alzheimer's disease are characterized by a mushroom spine loss that is rescued after the overexpression of TRPC6 channels or after a hyperforin treatment (H. Zhang et al., 2016). Hence, amyloid beta peptide 1-42 ( $A\beta_{1-42}$ ) oligomers downregulate the store-operated  $Ca^{2+}$  entry (SOCE), a prominent neuronal  $Ca^{2+}$  route (Bouron, 2023). The amplitude of SOCE is however restored when preincubating hippocampal neurons with 0.3  $\mu$ M hyperforin (Popugaeva et al., 2019). The intracerebral injection of  $A\beta$  fibrils increases the production of reactive oxygen species (ROS), causes an astroglial response and triggers neuronal cell death (Dinamarca et al., 2006). These responses are alleviated by hyperforin. Treating hippocampal neurons in culture with  $A\beta$  oligomers stimulates the production of ROS that is blunted by hyperforin (1  $\mu$ M). This finding is in line with previous reports showing that this bioactive molecule possesses antioxidant properties (Heilmann, Winkelmann, & Sticher, 2003). For instance, hyperforin inhibits the production of ROS in human isolated polymorphonuclear leukocytes (Feisst & Werz, 2004). In addition, hyperforin (1  $\mu$ M) reduces the generation of ROS induced by  $A\beta$  in primary hippocampal neurons (Dinamarca et al., 2008).

Aluminium maltolate (Almal) decreases the viability of PC12 cells and increases protein expression of key factors of Alzheimer's disease such as APP, BACE,  $A\beta_{1-42}$ . Pretreating cells with hyperforin (1  $\mu$ M/12 h) reverses these responses (Huang et al., 2017). Almal also decreases cell viability of SH-SY5Y cells and causes a loss of  $\Delta\psi_m$  (H. Wang et al., 2019). However, pre-incubating SH-SY5Y cells with low concentrations of hyperforin (0.25 – 2.5  $\mu$ M) for 12 or 24h exerts a neuroprotective effect by attenuating the Almal-induced cell death, LDH-release, ROS production, and  $\Delta\psi_m$  loss, indicating that hyperforin can alleviate mitochondrial dysfunctions. Taken together, these studies show that hyperforin exerts neuroprotective actions against oxidative damages (Huang et al., 2017) (H. Wang et al., 2019).

In a rat model of status epilepticus (a type of continuous seizure activity), the intraperitoneal injection (i.p.) of pilocarpine causes a microglial activation, p65 NF- $\kappa$ B phosphorylation, and augments tumour necrosis factor (TNF) expression (Lee, Kim, Kim, Kim, & Kang, 2014). However, in hyperforin-treated animals (3  $\mu$ M/1 week) there is a reduction of neuronal damages compared to vehicle-treated rats. This indicates that *in vivo* hyperforin can attenuate microglial activation and prevent the development of neuro-inflammatory responses in the brain. Hyperforin was also shown to suppress the inflammatory responses of glial cells. The infection of murine primary mixed glia (astrocytes and microglia) with *Toxoplasma gondii* induces an inflammatory response characterized by higher levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  (Shinjyo et al., 2021). At concentrations above 1  $\mu$ M hyperforin inhibits the growth of the parasite in glial cells and suppresses the induction of cytokines. Hyperforin (1  $\mu$ M)

restores the relative number of neurons (Shinjyo et al., 2021), suggesting that the molecule exerts a positive role against the infection-induced inflammation in neuroglia.

Protective effects of hyperforin have been observed in non-neuronal cells like pancreatic  $\beta$  cells. In this cell type, hyperforin prevents the cytokine-induced apoptotic cell death by inhibiting various deleterious intracellular signaling pathways (e.g. NF- $\kappa$ B- and MAPK-dependent pathways) (Novelli et al., 2016). It also reduces the extent of apoptotic cell death caused by an acute brain ischemic injury (Lin et al., 2013). In addition, hyperforin has a beneficial effect in stroke as well as in post-stroke depression (PSD) and post-stroke anxiety (PSA), two recognized complications of stroke. For instance, in a model of transient middle cerebral artery occlusion in mice, hyperforin promotes neuroangiogenesis and neurogenesis in the subventricular zone. It also improves recovery after ischemic stroke (Yao et al., 2019). In a mice model of stroke induced by middle cerebral artery occlusion, hyperforin treatment improves PSD and PSA (Y. Zhang et al., 2019). It also stimulates hippocampal neurogenesis and memory function. These studies emphasise a beneficial post-stroke effect of hyperforin, at least in mice (Yao et al., 2019) (Y. Zhang et al., 2019). Altogether, these *in vitro* and *in vivo* data underline the beneficial neuroprotective properties of hyperforin.

When considering the putative interest of hyperforin to cure or alleviate symptoms of neurodegenerative diseases characterized by aggregates of proteins, it is important to mention its action on autophagy, a vital process participating in the degradation of misfolded or aggregated proteins as well as in the clearance of damaged organelles. Dysregulation of autophagy is thought to play a role in various neurologic and neurodegenerative disorders (Klionsky et al., 2021) (Frake, Ricketts, Menzies, & Rubinsztein, 2015). Tetrahydrohyperforin activates the autophagic degradation of APP via a mechanism dependent on mTOR activity and Atg5 (Cavieres et al., 2015). Moreover, tetrahydrohyperforin enhances the formation of autophagosomes and the protein levels of the autophagy-related proteins LC3, beclin-1 and Atg5 (J. Zhang et al., 2018). In rats, early maternal separation induces a depressive-like behavior associated with a reduced expression of the autophagy marker beclin-3, and a decreased LC3II/I ratio. A treatment with hyperforin alleviates these effects, further suggesting that it interferes with the autophagy process (S. Wang et al., 2021). It is worthy of note that synthetic antidepressant molecules such as fluoxetine, clomipramine and amitriptyline are also known to interfere with the autophagic flux (Gulbins et al., 2018) (Cavaliere et al., 2019). Collectively, these studies show that hyperforin has neuroprotective and anti-inflammatory properties, and enhances the degradation of proteins via its impact on the autophagy process, making this component of SJW extracts a promising clinically relevant molecule. However, one major concern regarding the pharmacological profile of hyperforin is its interference with drugs via its action on the pregnane X receptor (PXR) and the cytochrome P450 3A4 monooxygenase, an enzyme involved in the metabolism of many drugs (Moore et al., 2000) (Chrubasik-Hausmann, Vlachojannis, & McLachlan, 2019). This interaction has clinical implication because it may cause unwanted side

effects. The development of hyperforin-derived molecules lacking drug-drug interactions would provide promising pharmacological tools of clinical interest. In this context, the newly synthesized hyperforin analog Hyp13, that does not bind PXR, represents a very attractive molecule (El Hamdaoui et al., 2022).

### **Conclusions and future directions**

Studies aiming at identifying the therapeutically active antidepressant constituents of SJW extracts have underscored the role of hyperforin. This lipophilic molecule influences the activity of a wide range of cellular targets (Figure 1). Although discrepant results have been published, hyperforin enhances TRPC6 activity without influencing other TRPC channels. Several groups took advantage of this peculiar pharmacological property to uncover the functions of native TRPC6 channels. Overall, *in vitro* studies indicate that hyperforin is a multi-target drug perturbing the cellular homeostasis of cations, principally  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Zn}^{2+}$  and  $\text{H}^+$ . So far, the patho-physiological relevance of these actions is currently unknown. A clear understanding of the molecular and cellular antidepressant actions of hyperforin is missing. Further work is needed to examine more closely the cellular neurobiology of this bioactive molecule. For instance, clarifying the functional relevance of the protonophore properties of hyperforin is necessary to fully understand the clinical implications. It is unknown whether other biologically relevant ions such as copper, iron, magnesium or chloride are affected by hyperforin. This latter point is a promising area for future fruitful studies.

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**Legend of Figure 1** The schematic summarizes some of the main neuro-cellular effects of hyperforin discussed in the manuscript. Hyperforin crosses phospholipid membranes and influences their fluidity and rigidity ①. In addition, it interferes with internal compartments and organelles like mitochondria ② that are major intracellular targets. Hyperforin promotes the release of cations ( $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{H}^+$ ) from these organelles ③. As a protonophore, it is also likely to release  $\text{H}^+$  from any intracellular acidic vesicular compartment. Hyperforin impairs  $\Delta\psi_m$  and influences the mitochondrial production of ROS, respiration and some mitochondrial electron transport chain proteins ④. Hyperforin was also reported to enhance the activity of the  $\text{Na}^+/\text{H}^+$  exchanger and of the cationic channel TRPC6 that transports  $\text{Ca}^{2+}$  and  $\text{Na}^+$  ions ⑤. However, hyperforin has been described as a blocker of various ion channels of the cell surface and of the choline transporter ⑥. Chronically applied, it induces the internalization of  $\beta 1$ -adrenergic receptors ( $\beta 1$ -AR) ⑦. Hyperforin regulates the degradation of proteins via its action on the autophagy process ⑧ and favours an unspecific neurotransmitter release ⑨.



**Table 1** This table summarizes *in vivo* experiments illustrating the antidepressant properties of hyperforin.

<b>Animal model</b>	<b>Dosage</b>	<b>Treatment</b>	<b>Effects</b>	<b>References</b>
Sprague Dawley rats	1 mg/kg	Single dose	Anxiolytic-like activity	(S. S. Chatterjee, Noldner, Koch, & Erdelmeier, 1998)
Sprague Dawley rats	50, 150, 300 mg/kg/day	3 days	Antidepressant-like effect	(S. S. Chatterjee, Bhattacharya, et al., 1998)
Sprague Dawley rats	20, 50, 60 300 mg/kg/day	3 or 9 days	Antidepressant-like effect	(S. S. Chatterjee, Noldner, et al., 1998)
Sprague Dawley rats	0.3, 1 and 3 mg/kg	Single injection	Anxiolytic-like activity	(S. S. Chatterjee, Noldner, et al., 1998)
Wister rats	20 mg/kg/day	Intragastric administration for 21 days	improves spatial cognition and synaptic plasticity; influences dendritic length density and number.	(Y. Liu, Liu, Qin, Zhu, & Yang, 2015)
Wister rats	3 mg/kg/day	Intragastric administration for 2 weeks	decreases anxiety-like activity.	(Zhu, Liu, Qin, & Yang, 2015)
C57BL/6J mice	1, 2.5, 5, and 10 mg/kg	Single intraperitoneal (i.p.) injection	antidepressant effects via a mechanism sensitive to TRPC6 channels blockers and inhibitors of the Akt kinase.	(Pochwat et al., 2018)
C57Bl6 mice	2.5 and 5 mg/kg	daily (i.p.) injection for 9 days	increases Zn concentration (hippocampus); Increases BDNF levels (hippocampus).	(Szewczyk et al., 2019)

**Table 2** Overview of the effects of hyperforin on memory and potential implication in Alzheimer's disease.

<b>Animal model</b>	<b>dosage</b>	<b>Treatment</b>		<b>References</b>
Wister rats	1.25 and 2.5 mg/kg/day	1-7 days	Improves learning ability	(Klusa et al., 2001)
BALB/c mice	1.25 and 2.5 mg/kg	1 days	Improves memory acquisition and consolidation	(Klusa et al., 2001)
Sprague-Dawley rats	Single intra-hippocampal injection of 6 $\mu$ M hyperforin	4 days later	Protects against A $\beta$ -induced toxicity, neuroinflammatory responses and cognitive impairments	(Dinamarca et al., 2006)
Double transgenic APP <sub>SWE</sub> /PS1 <sup><math>\Delta</math>E9</sup> mice	i.p. injection of 6 mg/kg IDN5706 (tetrahydrohyperforin)	Three times a week for 10 weeks	Improves memory deficit, diminishes A $\beta$ plaque size, attenuates oxidative damages	(Cerpa et al., 2010)
Double transgenic APP <sub>SWE</sub> /PS1 <sup><math>\Delta</math>E9</sup> mice	i.p. injection of 4 mg/kg IDN5706 (tetrahydrohyperforin)	10 weeks	IDN 5706 reduces spatial memory impairments; reduction of tau hyperphosphorylation Prevention of synaptic protein loss.	(Inestrosa et al., 2011)
Double transgenic APP <sub>SWE</sub> /PS1 <sup><math>\Delta</math>E9</sup> mice	i.p. injection of 4 mg/kg IDN5706 (tetrahydrohyperforin)	Three times a week for 10 weeks	Stimulates hippocampal neurogenesis, improves long-term spatial memory.	Abbott et al, 2013
C57Bl/6 mice	i.p. injection of 6 mg/kg IDN5706 (tetrahydrohyperforin)	Three times a week for 10 weeks	Improves performance in memory test via TRPC3/6/7 channels	(Montecinos-Oliva, Schuller, Parodi, Melo, & Inestrosa, 2014)

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