



HAL
open science

Cellular neurobiology of hyperforin

Alexandre Bouron

► **To cite this version:**

Alexandre Bouron. Cellular neurobiology of hyperforin. *Phytotherapy Research*, 2024, 38 (2), pp.636-645. 10.1002/ptr.8063 . hal-04286229

HAL Id: hal-04286229

<https://hal.science/hal-04286229v1>

Submitted on 15 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Cellular neurobiology of hyperforin

Alexandre Bouron

Université Grenoble Alpes, CNRS, CEA, Inserm UA13 BGE, 38000 Grenoble, France.

Address: Inserm UA13

Genetics and Chemogenomics Lab

CEA

17 rue des Martyrs

38054 Grenoble cedex 9 – France

Tel +33.438.785.258 ; Fax +33.438.785.051 ; Email alexandre.bouron@cea.fr

Data availability statement : The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding : This work was not supported by specific fundings but the author received support from the Centre National de la Recherche Scientifique (CNRS), Commissariat à l’Energie Atomique et aux Energies Alternatives (CEA), Institut national de la Santé et de la Recherche Médicale (Inserm), and Université de Grenoble Alpes (UGA).

Author contribution: AB collected and analyzed the data, wrote the manuscript.

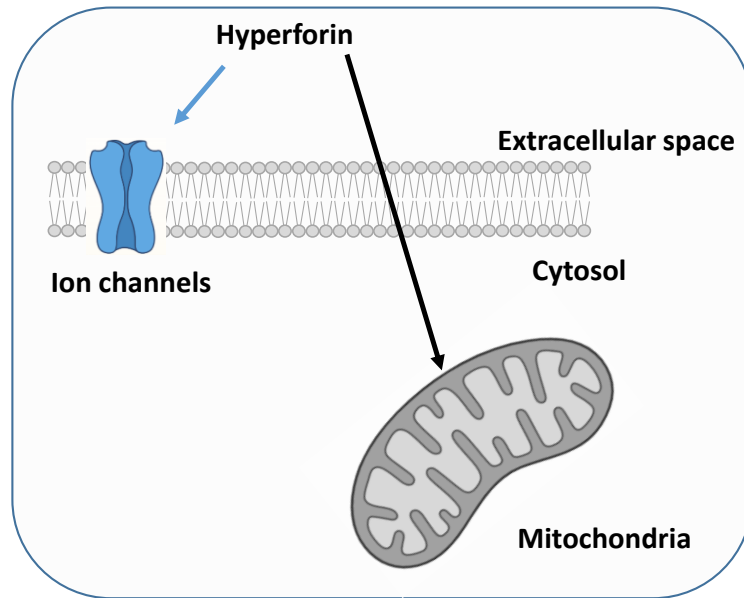
Conflict of interest: None.

Ethics approval statement: not relevant.

Patient consent statement : not relevant.

Permission to reproduce material from other sources: not relevant.

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 Ca^{2+} , Na^+ , Zn^{2+} and 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.

Table of content

Introduction	5
1. Hyperforin and membrane proteins.....	6
2. Cytotoxicity of hyperforin.....	9
3. Hyperforin and mood disorders.....	10
4. Hyperforin and Alzheimer's disease	10
Conclusions and future directions	13

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 μM) 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 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 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 μM hyperforin inhibits partially and reversibly all the voltage-gated ion currents tested (Na^+ , K^+ , and 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 μM hyperforin shift the I/V curves and reversibly depress the amplitude of voltage-gated Ca^{2+} currents of P-type (Fisunov et al., 2000). Contrasting results have been obtained concerning the effects of hyperforin on NMDA receptors. Ca^{2+} imaging experiments showed that hyperforin (10 μM) inhibits NMDA-induced 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 IC_{50} of $\approx 3 \mu\text{M}$ (S. Chatterjee et al., 1999). However, a concentration of 1 μM 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 (Na^+) ionophore by itself elevates the free intracellular Na^+ concentration (Singer et al., 1999). Contrasting results have been obtained regarding the contribution of the Na^+/H^+ exchanger. Hyperforin was thought to stimulate the activity of the Na^+/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 Ca^{2+} and Na^+ ions, is enhanced by hyperforin (see below) and may also contribute to these hyperforin-triggered intracellular Na^+ rises. Of note, the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (Treiber et al., 2005) as well as the Na^+/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 Ca^{2+} and Na^+ ions with an 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 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). Ca^{2+} imaging experiments confirmed that, acutely applied, hyperforin evokes 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 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 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 (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 Ca^{2+} and zinc (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 (⁷⁷⁷LLKL⁷⁸⁰) (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) Ca²⁺ entry through TRPC6. Thus, the hyperforin-induced TRPC6-dependent Ca²⁺ 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 β 1-adrenergic receptors (β 1-AR). For instance, a chronic hyperforin treatment causes the internalization of β 1-AR, reduces cAMP levels and β 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 μ 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 μ 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 μ M (H. Zhang et al., 2016), whereas a 24h treatment with 0.01-1

μM hyperforin has no effect on the viability of hippocampal neurons (Dinamarca, Cerpa, Garrido, Hancke, & Inestrosa, 2006). However, a 10 μM 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 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 Zn^{2+} from mitochondria (Tu et al., 2010), changes the Zn^{2+} storage capacity of the brain by upregulating the expression of the Zn^{2+} -binding proteins metallothioneins (Gibon, Richaud, & Bouron, 2011), and increases the concentration of 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 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 β -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 μM) 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 μ 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 μ 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 μ 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 μ 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 μ 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- κ B phosphorylation, and augments tumour necrosis factor (TNF) expression (Lee, Kim, Kim, Kim, & Kang, 2014). However, in hyperforin-treated animals (3 μ 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 β , IL-6, and TNF- α (Shinjyo et al., 2021). At concentrations above 1 μ M hyperforin inhibits the growth of the parasite in glial cells and suppresses the induction of cytokines. Hyperforin (1 μ 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 β cells. In this cell type, hyperforin prevents the cytokine-induced apoptotic cell death by inhibiting various deleterious intracellular signaling pathways (e.g. NF- κ 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 Ca^{2+} , Na^+ , Zn^{2+} and 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.

References

- Abbott, A. C., Calderon Toledo, C., Aranguiz, F. C., Inestrosa, N. C., & Varela-Nallar, L. (2013). Tetrahydrohyperforin increases adult hippocampal neurogenesis in wild-type and APP^{swe}/PS1^{DeltaE9} mice. *J Alzheimers Dis*, *34*(4), 873-885. doi:10.3233/JAD-121714
- Abramowitz, J., & Birnbaumer, L. (2009). Physiology and pathophysiology of canonical transient receptor potential channels. *Faseb J*, *23*(2), 297-328.
- Apaydin, E. A., Maher, A. R., Shanman, R., Booth, M. S., Miles, J. N., Sorbero, M. E., & Hempel, S. (2016). A systematic review of St. John's wort for major depressive disorder. *Syst Rev*, *5*(1), 148. doi:10.1186/s13643-016-0325-2
- Beerhues, L. (2006). Hyperforin. *Phytochemistry*, *67*(20), 2201-2207.
- Berna-Erro, A., Albarran, L., Dionisio, N., Redondo, P. C., Alonso, N., Gomez, L. J., . . . Rosado, J. A. (2014). The canonical transient receptor potential 6 (TRPC6) channel is sensitive to extracellular pH in mouse platelets. *Blood Cells Mol Dis*, *52*(2-3), 108-115. doi:10.1016/j.bcmd.2013.08.007
- Biber, A., Fischer, H., Romer, A., & Chatterjee, S. S. (1998). Oral bioavailability of hyperforin from hypericum extracts in rats and human volunteers. *Pharmacopsychiatry*, *31 Suppl 1*, 36-43. doi:10.1055/s-2007-979344
- Bilia, A. R., Gallori, S., & Vincieri, F. F. (2002). St. John's wort and depression: efficacy, safety and tolerability-an update. *Life Sci*, *70*(26), 3077-3096.
- Bouron, A. (2023). Neuronal Store-Operated Calcium Channels. *Mol Neurobiol*. doi:10.1007/s12035-023-03352-5
- Buchholzer, M. L., Dvorak, C., Chatterjee, S. S., & Klein, J. (2002). Dual modulation of striatal acetylcholine release by hyperforin, a constituent of St. John's wort. *J Pharmacol Exp Ther*, *301*(2), 714-719.
- Butterweck, V., & Schmidt, M. (2007). St. John's wort: role of active compounds for its mechanism of action and efficacy. *Wien Med Wochenschr*, *157*(13-14), 356-361.
- Cardile, A., Zanre, V., Campagnari, R., Asson, F., Addo, S. S., Orlandi, E., & Menegazzi, M. (2023). Hyperforin Elicits Cytostatic/Cytotoxic Activity in Human Melanoma Cell Lines, Inhibiting Pro-Survival NF-kappaB, STAT3, AP1 Transcription Factors and the Expression of Functional Proteins Involved in Mitochondrial and Cytosolic Metabolism. *Int J Mol Sci*, *24*(2). doi:10.3390/ijms24021263
- Cavaliere, F., Fornarelli, A., Bertan, F., Russo, R., Marsal-Cots, A., Morrone, L. A., . . . Nicotera, P. (2019). The tricyclic antidepressant clomipramine inhibits neuronal autophagic flux. *Sci Rep*, *9*(1), 4881. doi:10.1038/s41598-019-40887-x
- Cavieres, V. A., Gonzalez, A., Munoz, V. C., Yefi, C. P., Bustamante, H. A., Barraza, R. R., . . . Burgos, P. V. (2015). Tetrahydrohyperforin Inhibits the Proteolytic Processing of Amyloid

- Precursor Protein and Enhances Its Degradation by Atg5-Dependent Autophagy. *PLoS ONE*, *10*(8), e0136313. doi:10.1371/journal.pone.0136313
- Cerpa, W., Hancke, J. L., Morazzoni, P., Bombardelli, E., Riva, A., Marin, P. P., & Inestrosa, N. C. (2010). The hyperforin derivative IDN5706 occludes spatial memory impairments and neuropathological changes in a double transgenic Alzheimer's mouse model. *Curr Alzheimer Res*, *7*(2), 126-133. doi:10.2174/156720510790691218
- Cervo, L., Rozio, M., Ekalle-Soppo, C. B., Guiso, G., Morazzoni, P., & Caccia, S. (2002). Role of hyperforin in the antidepressant-like activity of *Hypericum perforatum* extracts. *Psychopharmacology (Berl)*, *164*(4), 423-428.
- Chatterjee, S., Filippov, V., Lishko, P., Maximyuk, O., Noldner, M., & Krishtal, O. (1999). Hyperforin attenuates various ionic conductance mechanisms in the isolated hippocampal neurons of rat. *Life Sci*, *65*(22), 2395-2405.
- Chatterjee, S. S., Bhattacharya, S. K., Wonnemann, M., Singer, A., & Muller, W. E. (1998). Hyperforin as a possible antidepressant component of hypericum extracts. *Life Sci*, *63*(6), 499-510.
- Chatterjee, S. S., Noldner, M., Koch, E., & Erdelmeier, C. (1998). Antidepressant activity of hypericum perforatum and hyperforin: the neglected possibility. *Pharmacopsychiatry*, *31 Suppl 1*, 7-15.
- Chen, S., Liu, X., Peng, C., Tan, C., Sun, H., Liu, H., . . . Liu, J. (2021). The phytochemical hyperforin triggers thermogenesis in adipose tissue via a Dlat-AMPK signaling axis to curb obesity. *Cell Metab*, *33*(3), 565-580 e567. doi:10.1016/j.cmet.2021.02.007
- Chiang, I. T., Chen, W. T., Tseng, C. W., Chen, Y. C., Kuo, Y. C., Chen, B. J., . . . Wang, W. S. (2017). Hyperforin Inhibits Cell Growth by Inducing Intrinsic and Extrinsic Apoptotic Pathways in Hepatocellular Carcinoma Cells. *Anticancer Res*, *37*(1), 161-167. doi:10.21873/anticancer.11301
- Chrubasik-Hausmann, S., Vlachoianis, J., & McLachlan, A. J. (2019). Understanding drug interactions with St John's wort (*Hypericum perforatum* L.): impact of hyperforin content. *J Pharm Pharmacol*, *71*(1), 129-138. doi:10.1111/jphp.12858
- Dinamarca, M. C., Arrazola, M., Toledo, E., Cerpa, W. F., Hancke, J., & Inestrosa, N. C. (2008). Release of acetylcholinesterase (AChE) from beta-amyloid plaques assemblies improves the spatial memory impairments in APP-transgenic mice. *Chem Biol Interact*, *175*(1-3), 142-149.
- Dinamarca, M. C., Cerpa, W., Garrido, J., Hancke, J. L., & Inestrosa, N. C. (2006). Hyperforin prevents beta-amyloid neurotoxicity and spatial memory impairments by disaggregation of Alzheimer's amyloid-beta-deposits. *Mol Psychiatry*, *11*(11), 1032-1048.
- Ding, F., Zhang, X., Li, X., Zhang, Y., Li, B., & Ding, J. (2014). Mammalian target of rapamycin complex 2 signaling pathway regulates transient receptor potential cation channel 6 in podocytes. *PLoS ONE*, *9*(11), e112972. doi:10.1371/journal.pone.0112972

- Ding, Y., Winters, A., Ding, M., Graham, S., Akopova, I., Muallem, S., . . . Ma, R. (2011). Reactive oxygen species-mediated TRPC6 protein activation in vascular myocytes, a mechanism for vasoconstrictor-regulated vascular tone. *J Biol Chem*, 286(36), 31799-31809.
- Eckert, G. P., Keller, J. H., Jourdan, C., Karas, M., Volmer, D. A., Schubert-Zsilavec, M., & Muller, W. E. (2004). Hyperforin modifies neuronal membrane properties in vivo. *Neurosci Lett*, 367(2), 139-143.
- Eckert, G. P., & Muller, W. E. (2001). Effects of hyperforin on the fluidity of brain membranes. *Pharmacopsychiatry*, 34 Suppl 1, S22-25. doi:10.1055/s-2001-15461
- El Hamdaoui, Y., Zheng, F., Fritz, N., Ye, L., Tran, M. A., Schwickert, K., . . . Friedland, K. (2022). Analysis of hyperforin (St. John's wort) action at TRPC6 channel leads to the development of a new class of antidepressant drugs. *Mol Psychiatry*, 27(12), 5070-5085. doi:10.1038/s41380-022-01804-3
- Feisst, C., & Werz, O. (2004). Suppression of receptor-mediated Ca²⁺ mobilization and functional leukocyte responses by hyperforin. *Biochem Pharmacol*, 67(8), 1531-1539.
- Feng, S., Li, H., Tai, Y., Huang, J., Su, Y., Abramowitz, J., . . . Wang, Y. (2013). Canonical transient receptor potential 3 channels regulate mitochondrial calcium uptake. *Proc Natl Acad Sci U S A*, 110(27), 11011-11016. doi:10.1073/pnas.1309531110
- Fisunov, A., Lozovaya, N., Tsintsadze, T., Chatterjee, S., Noldner, M., & Krishtal, O. (2000). Hyperforin modulates gating of P-type Ca²⁺ current in cerebellar Purkinje neurons. *Pflugers Arch*, 440(3), 427-434.
- Frake, R. A., Ricketts, T., Menzies, F. M., & Rubinsztein, D. C. (2015). Autophagy and neurodegeneration. *J Clin Invest*, 125(1), 65-74. doi:10.1172/JCI73944
- Froestl, B., Steiner, B., & Muller, W. E. (2003). Enhancement of proteolytic processing of the beta-amyloid precursor protein by hyperforin. *Biochem Pharmacol*, 66(11), 2177-2184.
- Gibon, J., Deloulme, J. C., Chevallier, T., Ladeveze, E., Abrous, D. N., & Bouron, A. (2013). The antidepressant hyperforin increases the phosphorylation of CREB and the expression of TrkB in a tissue-specific manner. *Int J Neuropsychopharmacol*, 16(1), 189-198.
- Gibon, J., Richaud, P., & Bouron, A. (2011). Hyperforin changes the zinc-storage capacities of brain cells. *Neuropharmacology*, 61, 1321-1326.
- Griesi-Oliveira, K., Acab, A., Gupta, A. R., Sunaga, D. Y., Chailangkarn, T., Nicol, X., . . . Muotri, A. R. (2015). Modeling non-syndromic autism and the impact of TRPC6 disruption in human neurons. *Mol Psychiatry*, 20(11), 1350-1365. doi:10.1038/mp.2014.141
- Griffith, T. N., Varela-Nallar, L., Dinamarca, M. C., & Inestrosa, N. C. (2010). Neurobiological effects of Hyperforin and its potential in Alzheimer's disease therapy. *Curr Med Chem*, 17(5), 391-406.

- Gulbins, A., Schumacher, F., Becker, K. A., Wilker, B., Soddemann, M., Boldrin, F., . . . Gulbins, E. (2018). Antidepressants act by inducing autophagy controlled by sphingomyelin-ceramide. *Mol Psychiatry*, *23*(12), 2324-2346. doi:10.1038/s41380-018-0090-9
- Heilmann, J., Winkelmann, K., & Sticher, O. (2003). Studies on the antioxidative activity of phloroglucinol derivatives isolated from hypericum species. *Planta Med*, *69*(3), 202-206. doi:10.1055/s-2003-38477
- Heiser, J. H., Schuwald, A. M., Sillani, G., Ye, L., Muller, W. E., & Leuner, K. (2013). TRPC6 channel-mediated neurite outgrowth in PC12 cells and hippocampal neurons involves activation of RAS/MEK/ERK, PI3K, and CAMKIV signaling. *J Neurochem*, *127*(3), 303-313. doi:10.1111/jnc.12376
- Herraiz, T., & Guillen, H. (2018). Monoamine Oxidase-A Inhibition and Associated Antioxidant Activity in Plant Extracts with Potential Antidepressant Actions. *Biomed Res Int*, *2018*, 4810394. doi:10.1155/2018/4810394
- Hoffmann, M., Lopez, J. J., Pergola, C., Feisst, C., Pawelczik, S., Jakobsson, P. J., . . . Werz, O. (2010). Hyperforin induces Ca(2+)-independent arachidonic acid release in human platelets by facilitating cytosolic phospholipase A(2) activation through select phospholipid interactions. *Biochim Biophys Acta*, *1801*(4), 462-472.
- Hostanska, K., Reichling, J., Bommer, S., Weber, M., & Saller, R. (2003). Hyperforin a constituent of St John's wort (*Hypericum perforatum* L.) extract induces apoptosis by triggering activation of caspases and with hypericin synergistically exerts cytotoxicity towards human malignant cell lines. *Eur J Pharm Biopharm*, *56*(1), 121-132.
- Hsu, F. T., Chen, W. T., Wu, C. T., & Chung, J. G. (2020). Hyperforin induces apoptosis through extrinsic/intrinsic pathways and inhibits EGFR/ERK/NF-kappaB-mediated anti-apoptotic potential in glioblastoma. *Environ Toxicol*, *35*(10), 1058-1069. doi:10.1002/tox.22942
- Huang, W., Cheng, P., Yu, K., Han, Y., Song, M., & Li, Y. (2017). Hyperforin attenuates aluminum-induced Abeta production and Tau phosphorylation via regulating Akt/GSK-3beta signaling pathway in PC12 cells. *Biomed Pharmacother*, *96*, 1-6. doi:10.1016/j.biopha.2017.09.114
- Inestrosa, N. C., Tapia-Rojas, C., Griffith, T. N., Carvajal, F. J., Benito, M. J., Rivera-Dictter, A., . . . Varela-Nallar, L. (2011). Tetrahydrohyperforin prevents cognitive deficit, Abeta deposition, tau phosphorylation and synaptotoxicity in the APP^{swe}/PSEN1^{DeltaE9} model of Alzheimer's disease: a possible effect on APP processing. *Transl Psychiatry*, *1*, e20. doi:10.1038/tp.2011.19
- Jakobs, D., Hage-Hulsmann, A., Prenner, L., Kolb, C., Weiser, D., & Haberlein, H. (2013). Downregulation of beta1 -adrenergic receptors in rat C6 glioblastoma cells by hyperforin and hyperoside from St John's wort. *J Pharm Pharmacol*, *65*(6), 907-915. doi:10.1111/jphp.12050
- Jia, Y., Zhou, J., Tai, Y., & Wang, Y. (2007). TRPC channels promote cerebellar granule neuron survival. *Nat Neurosci*, *10*(5), 559-567.

- Keksel, N., Bussmann, H., Unger, M., Drewe, J., Boonen, G., Haberlein, H., & Franken, S. (2019). St John's wort extract influences membrane fluidity and composition of phosphatidylcholine and phosphatidylethanolamine in rat C6 glioblastoma cells. *Phytomedicine*, *54*, 66-76.
doi:10.1016/j.phymed.2018.06.013
- Klionsky, D. J., Petroni, G., Amaravadi, R. K., Baehrecke, E. H., Ballabio, A., Boya, P., . . . Pietrocola, F. (2021). Autophagy in major human diseases. *Embo J*, *40*(19), e108863.
doi:10.15252/emj.2021108863
- Klusa, V., Germane, S., Noldner, M., & Chatterjee, S. S. (2001). Hypericum extract and hyperforin: memory-enhancing properties in rodents. *Pharmacopsychiatry*, *34 Suppl 1*, S61-69.
- Ko, A. R., & Kang, T. C. (2017). TRPC6-mediated ERK1/2 phosphorylation prevents dentate granule cell degeneration via inhibiting mitochondrial elongation. *Neuropharmacology*, *121*, 120-129.
doi:10.1016/j.neuropharm.2017.05.004
- Koch, E., & Chatterjee, S. S. (2001). Hyperforin stimulates intracellular calcium mobilisation and enhances extracellular acidification in DDT1-MF2 smooth muscle cells. *Pharmacopsychiatry*, *34 Suppl 1*, S70-73.
- Komoroski, B. J., Zhang, S., Cai, H., Hutzler, J. M., Frye, R., Tracy, T. S., . . . Venkataramanan, R. (2004). Induction and inhibition of cytochromes P450 by the St. John's wort constituent hyperforin in human hepatocyte cultures. *Drug Metab Dispos*, *32*(5), 512-518.
- Lee, S. K., Kim, J. E., Kim, Y. J., Kim, M. J., & Kang, T. C. (2014). Hyperforin attenuates microglia activation and inhibits p65-Ser276 NFkappaB phosphorylation in the rat piriform cortex following status epilepticus. *Neurosci Res*, *85*, 39-50. doi:10.1016/j.neures.2014.05.006
- Leuner, K., Kazanski, V., Muller, M., Essin, K., Henke, B., Gollasch, M., . . . Muller, W. E. (2007). Hyperforin--a key constituent of St. John's wort specifically activates TRPC6 channels. *Faseb J*, *21*(14), 4101-4111.
- Leuner, K., Li, W., Amaral, M. D., Rudolph, S., Calfa, G., Schuwald, A. M., . . . Pozzo-Miller, L. (2013). Hyperforin modulates dendritic spine morphology in hippocampal pyramidal neurons by activating Ca(2+) -permeable TRPC6 channels. *Hippocampus*, *23*(1), 40-52.
doi:10.1002/hipo.22052
- Lin, Y., Zhang, J. C., Fu, J., Chen, F., Wang, J., Wu, Z. L., & Yuan, S. Y. (2013). Hyperforin attenuates brain damage induced by transient middle cerebral artery occlusion (MCAO) in rats via inhibition of TRPC6 channels degradation. *J Cereb Blood Flow Metab*, *33*(2), 253-262.
doi:10.1038/jcbfm.2012.164
- Linde, K., Ramirez, G., Mulrow, C. D., Pauls, A., Weidenhammer, W., & Melchart, D. (1996). St John's wort for depression--an overview and meta-analysis of randomised clinical trials. *Bmj*, *313*(7052), 253-258.

- Liu, J. Y., Liu, Z., Wang, D. M., Li, M. M., Wang, S. X., Wang, R., . . . Yang, D. P. (2011). Induction of apoptosis in K562 cells by dicyclohexylammonium salt of hyperforin through a mitochondrial-related pathway. *Chem Biol Interact*, *190*(2-3), 91-101.
- Liu, Y., Liu, C., Qin, X., Zhu, M., & Yang, Z. (2015). The change of spatial cognition ability in depression rat model and the possible association with down-regulated protein expression of TRPC6. *Behav Brain Res*, *294*, 186-193. doi:10.1016/j.bbr.2015.07.062
- Marsh, W. L., & Davies, J. A. (2002). The involvement of sodium and calcium ions in the release of amino acid neurotransmitters from mouse cortical slices elicited by hyperforin. *Life Sci*, *71*(22), 2645-2655.
- Medina, M. A., Martinez-Poveda, B., Amores-Sanchez, M. I., & Quesada, A. R. (2006). Hyperforin: more than an antidepressant bioactive compound? *Life Sci*, *79*(2), 105-111.
- Menegazzi, M., Masiello, P., & Novelli, M. (2020). Anti-Tumor Activity of Hypericum perforatum L. and Hyperforin through Modulation of Inflammatory Signaling, ROS Generation and Proton Dynamics. *Antioxidants (Basel)*, *10*(1). doi:10.3390/antiox10010018
- Merhi, F., Tang, R., Piedfer, M., Mathieu, J., Bombarda, I., Zaher, M., . . . Bauvois, B. (2011). Hyperforin inhibits akt1 kinase activity and promotes caspase-mediated apoptosis involving bad and noxa activation in human myeloid tumor cells. *PLoS ONE*, *6*(10), e25963.
- Mitsopoulou, K. P., Vidali, V. P., Koliopoulos, G., Couladouros, E. A., & Michaelakis, A. (2014). Hyperforin and deoxycohumulone as a larvicidal agent against *Culex pipiens* (Diptera: Culicidae). *Chemosphere*, *100*, 124-129. doi:10.1016/j.chemosphere.2013.11.073
- Montecinos-Oliva, C., Schuller, A., Parodi, J., Melo, F., & Inestrosa, N. C. (2014). Effects of tetrahydrohyperforin in mouse hippocampal slices: neuroprotection, long-term potentiation and TRPC channels. *Curr Med Chem*, *21*(30), 3494-3506.
- Moore, L. B., Goodwin, B., Jones, S. A., Wisely, G. B., Serabjit-Singh, C. J., Willson, T. M., . . . Kliewer, S. A. (2000). St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. *Proc Natl Acad Sci U S A*, *97*(13), 7500-7502.
- Muller, M., Essin, K., Hill, K., Beschmann, H., Rubant, S., Schempp, C. M., . . . Leuner, K. (2008). Specific TRPC6 channel activation, a novel approach to stimulate keratinocyte differentiation. *J Biol Chem*, *283*(49), 33942-33954.
- Ng, Q. X., Venkatanarayanan, N., & Ho, C. Y. (2017). Clinical use of Hypericum perforatum (St John's wort) in depression: A meta-analysis. *J Affect Disord*, *210*, 211-221. doi:10.1016/j.jad.2016.12.048
- Nilius, B., & Szallasi, A. (2014). Transient receptor potential channels as drug targets: from the science of basic research to the art of medicine. *Pharmacol Rev*, *66*(3), 676-814. doi:10.1124/pr.113.008268
- Novelli, M., Menegazzi, M., Beffy, P., Porozov, S., Gregorelli, A., Giacomelli, D., . . . Masiello, P. (2016). St. John's wort extract and hyperforin inhibit multiple phosphorylation steps of cytokine

- signaling and prevent inflammatory and apoptotic gene induction in pancreatic beta cells. *Int J Biochem Cell Biol*, 81(Pt A), 92-104. doi:10.1016/j.biocel.2016.10.017
- Nowak, G., Szewczyk, B., & Pilc, A. (2005). Zinc and depression. An update. *Pharmacol Rep*, 57(6), 713-718.
- Orth, H. C., Rentel, C., & Schmidt, P. C. (1999). Isolation, purity analysis and stability of hyperforin as a standard material from *Hypericum perforatum* L. *J Pharm Pharmacol*, 51(2), 193-200. doi:10.1211/0022357991772132
- Pia Schiavone, B. I., Verotta, L., Rosato, A., Marilena, M., Gibbons, S., Bombardelli, E., . . . Corbo, F. (2014). Anticancer and Antibacterial Activity of Hyperforin and Its Derivatives. *Anticancer Agents Med Chem*.
- Pochwat, B., Szewczyk, B., Kotarska, K., Rafalo-Ulinska, A., Siwiec, M., Sowa, J. E., . . . Nowak, G. (2018). Hyperforin Potentiates Antidepressant-Like Activity of Lanicemine in Mice. *Front Mol Neurosci*, 11, 456. doi:10.3389/fnmol.2018.00456
- Popugaeva, E., Chernyuk, D., Zhang, H., Postnikova, T. Y., Pats, K., Fedorova, E., . . . Bezprozvanny, I. (2019). Derivatives of Piperazines as Potential Therapeutic Agents for Alzheimer's Disease. *Mol Pharmacol*, 95(4), 337-348. doi:10.1124/mol.118.114348
- Rothley, M., Schmid, A., Thiele, W., Schacht, V., Plaumann, D., Gartner, M., . . . Sleeman, J. P. (2009). Hyperforin and aristoforin inhibit lymphatic endothelial cell proliferation in vitro and suppress tumor-induced lymphangiogenesis in vivo. *Int J Cancer*, 125(1), 34-42.
- Roz, N., & Rehavi, M. (2003). Hyperforin inhibits vesicular uptake of monoamines by dissipating pH gradient across synaptic vesicle membrane. *Life Sci*, 73(4), 461-470. doi:10.1016/s0024-3205(03)00295-9
- Schafer, A. M., Bock, T., & Meyer Zu Schwabedissen, H. E. (2018). Establishment and Validation of Competitive Counterflow as a Method To Detect Substrates of the Organic Anion Transporting Polypeptide 2B1. *Mol Pharm*, 15(12), 5501-5513. doi:10.1021/acs.molpharmaceut.8b00631
- Schafer, A. M., Potterat, O., Seibert, I., Fertig, O., & Meyer Zu Schwabedissen, H. E. (2019). Hyperforin-Induced Activation of the Pregnane X Receptor Is Influenced by the Organic Anion-Transporting Polypeptide 2B1. *Mol Pharmacol*, 95(3), 313-323. doi:10.1124/mol.118.114066
- Schempp, C. M., Kirkin, V., Simon-Haarhaus, B., Kersten, A., Kiss, J., Termeer, C. C., . . . Simon, J. C. (2002). Inhibition of tumour cell growth by hyperforin, a novel anticancer drug from *St. John's wort* that acts by induction of apoptosis. *Oncogene*, 21(8), 1242-1250.
- Scheuble, J., Rossler, O. G., Ulrich, M., & Thiel, G. (2020). Pharmacological and genetic inhibition of TRPC6-induced gene transcription. *Eur J Pharmacol*, 886, 173357. doi:10.1016/j.ejphar.2020.173357
- Schulz, H. U., Schurer, M., Bassler, D., & Weiser, D. (2005). Investigation of pharmacokinetic data of hypericin, pseudohypericin, hyperforin and the flavonoids quercetin and isorhamnetin revealed

- from single and multiple oral dose studies with a hypericum extract containing tablet in healthy male volunteers. *Arzneimittelforschung*, 55(10), 561-568. doi:10.1055/s-0031-1296905
- Sell, T. S., Belkacemi, T., Flockerzi, V., & Beck, A. (2014). Protonophore properties of hyperforin are essential for its pharmacological activity. *Sci Rep*, 4, 7500. doi:10.1038/srep07500
- Shinjyo, N., Nakayama, H., Li, L., Ishimaru, K., Hikosaka, K., Suzuki, N., . . . Norose, K. (2021). Hypericum perforatum extract and hyperforin inhibit the growth of neurotropic parasite *Toxoplasma gondii* and infection-induced inflammatory responses of glial cells in vitro. *J Ethnopharmacol*, 267, 113525. doi:10.1016/j.jep.2020.113525
- Singer, A., Wonnemann, M., & Muller, W. E. (1999). Hyperforin, a major antidepressant constituent of St. John's Wort, inhibits serotonin uptake by elevating free intracellular Na⁺. *J Pharmacol Exp Ther*, 290(3), 1363-1368.
- Soni, H., & Adebisi, A. (2016). TRPC6 channel activation promotes neonatal glomerular mesangial cell apoptosis via calcineurin/NFAT and FasL/Fas signaling pathways. *Sci Rep*, 6, 29041. doi:10.1038/srep29041
- Spieß, D., Abegg, V. F., Chauveau, A., Rath, J., Treyer, A., Reinehr, M., . . . Simoes-Wüst, A. P. (2023). Transplacental passage of hyperforin, hypericin, and valerenic acid. *Front Pharmacol*, 14, 1123194. doi:10.3389/fphar.2023.1123194
- Swardfager, W., Herrmann, N., McIntyre, R. S., Mazereeuw, G., Goldberger, K., Cha, D. S., . . . Lanctot, K. L. (2013). Potential roles of zinc in the pathophysiology and treatment of major depressive disorder. *Neurosci Biobehav Rev*, 37(5), 911-929. doi:10.1016/j.neubiorev.2013.03.018
- Szewczyk, B., Pochwat, B., Muszynska, B., Opoka, W., Krakowska, A., Rafalo-Ulinska, A., . . . Nowak, G. (2019). Antidepressant-like activity of hyperforin and changes in BDNF and zinc levels in mice exposed to chronic unpredictable mild stress. *Behav Brain Res*, 372, 112045. doi:10.1016/j.bbr.2019.112045
- Takada, H., Furuya, K., & Sokabe, M. (2014). Mechanosensitive ATP release from hemichannels and Ca²⁺(+) influx through TRPC6 accelerate wound closure in keratinocytes. *J Cell Sci*, 127(Pt 19), 4159-4171. doi:10.1242/jcs.147314
- Thiel, G., & Rossler, O. G. (2017). Hyperforin activates gene transcription involving transient receptor potential C6 channels. *Biochem Pharmacol*, 129, 96-107. doi:10.1016/j.bcp.2017.01.007
- Treiber, K., Singer, A., Henke, B., & Muller, W. E. (2005). Hyperforin activates nonselective cation channels (NSCCs). *Br J Pharmacol*, 145(1), 75-83.
- Tu, P., Gibon, J., & Bouron, A. (2010). The TRPC6 channel activator hyperforin induces the release of zinc and calcium from mitochondria. *J Neurochem*, 112, 204-213.
- Wang, H., Shao, B., Yu, H., Xu, F., Wang, P., Yu, K., . . . Cao, Z. (2019). Neuroprotective role of hyperforin on aluminum maltolate-induced oxidative damage and apoptosis in PC12 cells and SH-SY5Y cells. *Chem Biol Interact*, 299, 15-26. doi:10.1016/j.cbi.2018.11.016

- Wang, S., Wang, S., Wang, Y., Xin, Y., Zhang, J., Yang, Z., & Liu, C. (2021). Hyperforin alleviates the psychiatric disorders of adult rats suffered from early maternal separation via activating autophagy. *Neurosci Lett*, *750*, 135750. doi:10.1016/j.neulet.2021.135750
- Wang, Y., Zhang, Y., He, J., Zhang, H., Xiao, L., Nazarali, A., . . . Li, X. M. (2011). Hyperforin promotes mitochondrial function and development of oligodendrocytes. *J Neurochem*, *119*(3), 555-568.
- Wiechmann, K., Muller, H., Fischer, D., Jauch, J., & Werz, O. (2015). The acylphloroglucinols hyperforin and myrtilleone A cause mitochondrial dysfunctions in leukemic cells by direct interference with mitochondria. *Apoptosis*, *20*(11), 1508-1517. doi:10.1007/s10495-015-1170-9
- Wofle, U., Seelinger, G., & Schempp, C. M. (2014). Topical application of St. John's wort (*Hypericum perforatum*). *Planta Med*, *80*(2-3), 109-120. doi:10.1055/s-0033-1351019
- Wonnemann, M., Singer, A., & Muller, W. E. (2000). Inhibition of synaptosomal uptake of 3H-L-glutamate and 3H-GABA by hyperforin, a major constituent of St. John's Wort: the role of amiloride sensitive sodium conductive pathways. *Neuropsychopharmacology*, *23*(2), 188-197.
- Yao, H., Zhang, Y., Shu, H., Xie, B., Tao, Y., Yuan, Y., . . . Zhang, J. (2019). Hyperforin Promotes Post-stroke Neuroangiogenesis via Astrocytic IL-6-Mediated Negative Immune Regulation in the Ischemic Brain. *Front Cell Neurosci*, *13*, 201. doi:10.3389/fncel.2019.00201
- Zanoli, P. (2004). Role of hyperforin in the pharmacological activities of St. John's Wort. *CNS Drug Rev*, *10*(3), 203-218.
- Zhang, H., Sun, S., Wu, L., Pchitskaya, E., Zakharova, O., Fon Tacer, K., & Bezprozvanny, I. (2016). Store-Operated Calcium Channel Complex in Postsynaptic Spines: A New Therapeutic Target for Alzheimer's Disease Treatment. *J Neurosci*, *36*(47), 11837-11850. doi:10.1523/JNEUROSCI.1188-16.2016
- Zhang, J., Wang, S., Rong, G., Cheng, F., Gui, B., & Shen, C. (2018). Tetrahydrohyperforin prevents articular cartilage degeneration and affects autophagy in rats with osteoarthritis. *Exp Ther Med*, *15*(6), 5261-5268. doi:10.3892/etm.2018.6098
- Zhang, Y., Yu, P., Liu, H., Yao, H., Yao, S., Yuan, S. Y., & Zhang, J. C. (2019). Hyperforin improves post-stroke social isolation-induced exaggeration of PSD and PSA via TGF-beta. *Int J Mol Med*, *43*(1), 413-425. doi:10.3892/ijmm.2018.3971
- Zhou, L. F., Chen, Q. Z., Yang, C. T., Fu, Z. D., Zhao, S. T., Chen, Y., . . . Li, J. H. (2018). TRPC6 contributes to LPS-induced inflammation through ERK1/2 and p38 pathways in bronchial epithelial cells. *Am J Physiol Cell Physiol*, *314*(3), C278-C288. doi:10.1152/ajpcell.00117.2017
- Zhu, M., Liu, C., Qin, X., & Yang, Z. (2015). Hyperforin alleviates mood deficits of adult rats suffered from early separation. *Neurosci Lett*, *608*, 1-5. doi:10.1016/j.neulet.2015.09.029

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 (Ca^{2+} , Zn^{2+} , and H^+) from these organelles ③. As a protonophore, it is also likely to release 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 Na^+/H^+ exchanger and of the cationic channel TRPC6 that transports Ca^{2+} and 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.

Animal model	Dosage	Treatment	Effects	References
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.

Animal model	dosage	Treatment		References
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 μ M hyperforin	4 days later	Protects against A β -induced toxicity, neuroinflammatory responses and cognitive impairments	(Dinamarca et al., 2006)
Double transgenic APP _{SWE} /PS1 ^{ΔE9} mice	i.p. injection of 6 mg/kg IDN5706 (tetrahydrohyperforin)	Three times a week for 10 weeks	Improves memory deficit, diminishes A β plaque size, attenuates oxidative damages	(Cerpa et al., 2010)
Double transgenic APP _{SWE} /PS1 ^{ΔE9} 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 _{SWE} /PS1 ^{ΔE9} 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)

Acknowledgments: The author wish to thank the support from the Centre National de la Recherche Scientifique (CNRS), Commissariat à l’Energie Atomique et aux Energies Alternatives (CEA), Institut National de la Santé et de la Recherche Médicale (Inserm), and Université de Grenoble Alpes (UGA).

Author contribution: AB collected and analyzed the data, wrote the manuscript.

Conflict of interest: None