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Hyperforin in St. John's Wort's Central Effects: What is the Mechanism of Action?

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ABSTRACT

The constituent(s) accounting for the antidepressant/anxiolytic, nootropic and anti-Alzheimer's properties of St John's wort (SJW) extracts is uncertain but the phloroglucinol hyperforin has been described as the main cause. Although its activities in humans have not yet been thoroughly investigated, it has antidepressant-like and anxiolytic-like effects in animal models, while *in vitro* studies suggest an interaction with the neurotransmitter systems thought to be involved in depression. Some of its actions on the neurotransmitter systems have also been proposed to explain the nootropic effects of SJW extracts, for which another possible explanation followed the *in vitro* observation that hyperforin activates transient receptor potential canonical 6 channels, which are vital for the formation of dendritic spines, plasticity and memory. *In vitro* hyperforin also has direct effects on amyloid-β (Aβ) fibrils, providing a potential mechanism for the antagonism of Aβ-induced neurotoxic effects and cognitive impairments. However, considering the low hyperforin brain uptake and concentrations reached in animals after pharmacologically effective doses of SJW extracts or authentic compound and its derivatives, its *in vivo* actions are unlikely to be due to direct interaction with acknowledged neurotransmitter transporters and receptors. Although it might interact with some central targets not yet evaluated *in vitro,* or even primarily act peripherally, thereby influencing central transmission, the central action may be due to one or more metabolites. Further neurochemical studies should therefore be extended to potential metabolite(s) which may have characteristic pharmacodynamic properties. Additional information is also needed on the brain uptake of hyperforin compared to its active metabolite(s). These data should enable us to identify the active principle (parent compound or its metabolite) and its target in the site of action, and undoubtedly help in extrapolating pharmacological findings across species.

Keywords: antidepressant- and anxiolytic-like effects, brain-to-blood distribution, *Hypericum perforatum* L. (St. John's wort), hyperforin, nootropic and anti-Alzheimer effects, neurotransmitter mechanisms and receptors, pharmacokinetics

Abbreviations: **Aβ**, amyloid-β; **AD**, Alzheimer's disease; **ACh**, acetylcholine; **AUC**, area under the plasma concentration-time curve; **BBB**, blood-brain barrier; $\mathbf{C_{max}}$, maximum concentrations; **CNS**, central nervous system; **CRF**, corticotropin-releasing factor; **CSF**, cerebrospinal fluid; **DA**, dopamine; **DCHA**, dicyclohexylammonium; **FST**, forced swimming test; **GABA**, gamma-aminobutyric acid; **5-HT**, serotonin; **IL6**, interleukin 6; **MAO-A**, monoamine oxidase A; **NPY**, neuropeptide-Y; **Pgp**, P-glycoprotein; **SJW**, St. John's wort; **Tg**, transgenic; **TMB**, trimethoxybenzoate; **TRPC6**, transient receptor potential canonical 6 channels

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INTRODUCTION

Hypericum perforatum L. (St. John's wort; SJW) has been known for a long time for its putative medicinal properties (Bombardelli and Morazzoni 1995) but nowadays its alcoholic extracts tend mainly to be used for some central nervous system (CNS) disorders (Greeson *et al.* 2001; Linde 2009; Sarris and Kavanagh 2009). The main indication, supported by randomized clinical trials (reviewed in (Clement *et al.* 2006; Linde *et al.* 2008) and studies in behavioural models in rodents (Oztürk 1997; Butterweck *et al.* 2001; Greeson *et al.* 2001; Müller 2003; Rodriguez-Landa and Contreras 2003; Butterweck and Schmidt 2007), is for the treatment of mild to moderate depression. SJW extracts have also been proposed, and in some countries are licensed, for the treatment of anxiety and sleep disorders, although such indications are only supported by preclinical evidence (Vandenbogaerde *et al.* 2000; Flausino *et al.* 2002; Beijamini and Andreatini 2003). Preclinical studies also suggest a potential for SJW extracts to combat tobacco and alcohol abuse (Uzbay 2008; Ruedeberg *et al.* 2009).

Some SJW extracts or components may have nootropic effects, improving spatial learning and memory (Khalifa 2001; Klusa *et al.* 2001; Widy-Tyszkiewicz *et al.* 2002; Trofimiuk and Braszko 2008). Neuroprotective effects of SJW and derivatives have been suggested too, also in models of Aβ-induced neurotoxicity (Lu *et al.* 2004; Silva *et al.* 2004).

It is still not clear which constituent(s) account, wholly or in part, for the different central actions of SJW extracts, and through what neurochemical mechanism(s). These ex-

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Invited Review

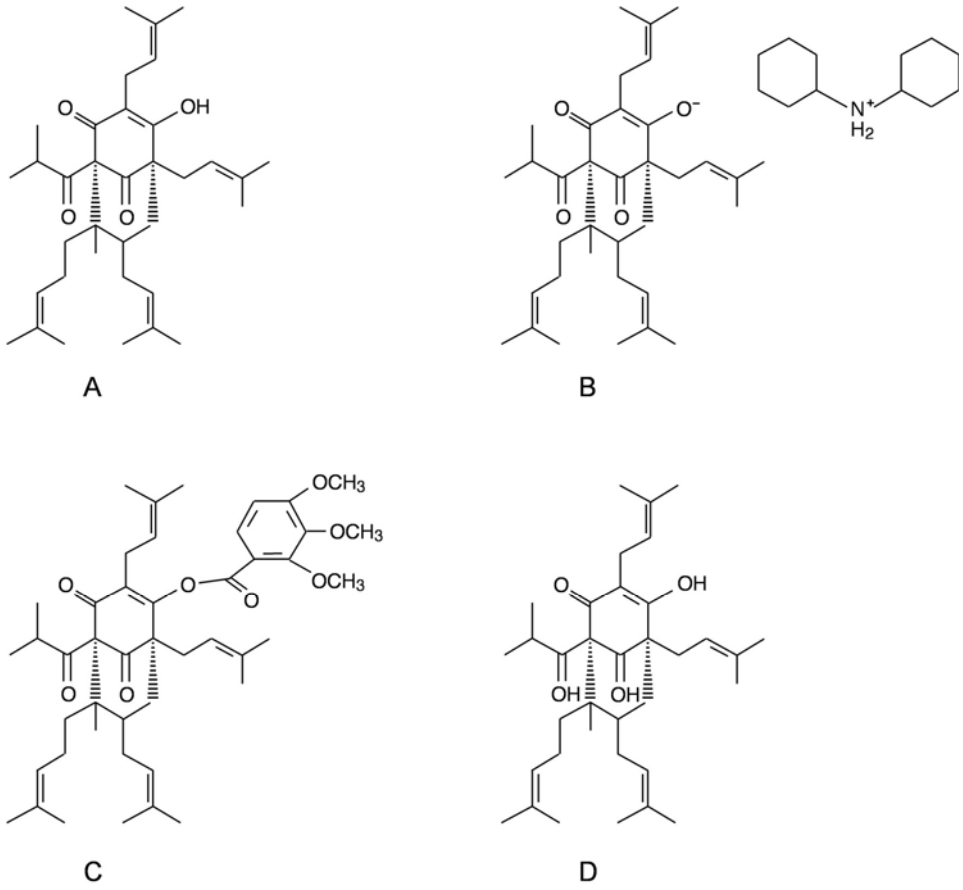

Fig. 1 Chemical structures in *Hypericum*. Hyperforin (**A**) and some of its more stable hyperforin derivatives: (**B**) hyperforin dicyclohexylammonium; (**C**) hyperforin trimethoxybenzoate; (**D**) tetrahydrohyperforin.

tracts contain acylphloroglucinols, flavonoids, naphthodianthrones, phenylpropanes, pronthocyanidines, xanthones, some amino acids and essential oils, whose concentrations and proportions in the plant are closely dependent on to the harvesting period, drying and storage conditions (Nahrstedt and Butterweck 1997; Jürgenliemk and Nahrstedt 2002; Tatsis *et al.* 2007). Antidepressant-like effects were ascribed at first to naphthodianthrones because of the MAO-inhibitory properties of hypericin. However, the concentrations active on this system (>100 µg/mL) are probably too high to be reached in the CNS after pharmacologically effective doses of the extracts, which contain about 0.3% of total naphthodianthrones (i.e. hypericins, pseudohypericin, their proto-derivatives and cyclopseudohypericin); and no effect on MAO activity was detected in rats given 100 mg/kg of whole extract intraperitoneally (see for reviews (Greeson *et al.* 2001; Mennini and Gobbi 2004).

Flavonoids, which account about 2-4% of the hydroalcolic extracts, were also considered because of the antidepressant-like activity seen in behavioral models of some derivatives (Butterweck *et al*. 2000; Butterweck and Schmidt 2007), but their wide distribution and higher concentrations in other plants suggests that they contribute to the overall effect of the extracts. Other components of SJW produce central effects or interact with central neurotransmitter transporters and receptors believed to be causally involved in psychiatric illness, including chlorogenic acid and its metabolite caffeic acid (Takeda *et al.* 2002, 2003) and the xanthone derivative 1,3,6,7-tetrahydroxyxanthone (Nahrstedt and Butterweck 1997). However, in view of their low concentrations in crude extracts (<1%) they should not have any real role in the central action of the extracts, although their pharmacokinetics or pharmacological activities in humans have not yet been thoroughly investigated.

Interest has increasingly focused on phloroglucinol derivatives, particularly hyperforin, the most abundant lipophilic compound of the hydroalcoholic extracts (Chatterjee *et al.* 1998a, 1998b; Laakmann *et al.* 1998; Jensen *et al.* 2001; Caccia and Gobbi 2009). Pure hyperforin showed antidepressant-like activity in behavioral models, and the antidepressant-like potencies of different extracts correlate with their hyperforin content (Chatterjee *et al.* 1998a, 1998b; Gambarana *et al.* 2001; Cervo *et al.* 2002, 2005). Most of the neuropharmacological properties of the extracts can also be demonstrated with pure hyperforin [see for reviews Greeson *et al.* (2001); Caccia and Gobbi (2009)]. Although this component is chemically unstable when exposed to light and air, making its pharmacological characterization and chemical analysis difficult (see for review (Caccia 2006), extraction of the plant material under controlled conditions gave extracts containing 2-5% hyperforin which could be maintained, appropriately stored, for long periods (Chatterjee *et al.* 1998b). Its salification with inorganic cations or ammonium salts (e.g. hyperforin dicyclohexylammonium, DCHA), esterification (e.g. hyperforin acetate and hyperforin trimethoxybenzoate, TMB) and reduction (tetrahydrohyperforin, octahydrohyperforin) leads to chemically stable derivatives (see also **Fig. 1**) which have already been used in pharmacological studies in rodents (Cervo *et al.* 2002; Zanoli *et al.* 2002; Cervo *et al.* 2005).

This review summarizes our current knowledge of the central actions of pure hyperforin and its more stable derivatives in relation to acknowledged neurochemical systems and receptors potentially involved in the pharmacological effects. The concentrations reached at the site of action after pharmacological doses of commercial extracts are also compared with the *in vitro* concentrations of the compounds affecting neurotransmitter mechanisms and receptors, and we discuss the importance of each mechanism in the central actions of the extracts.

PHARMACOLOGICAL PROFILE

Antidepressant-like effects have been detected after administration of pure hyperforin, its salts and esters and hyperforin-enriched CO_2 extract in different animal models (tail suspension test in mice, forced swimming test in rats, learned helplessness test in rats) (Chatterjee *et al.* 1998a, 1998b; Gambarana *et al.* 2001; Cervo *et al.* 2002, 2005). Doses of SJW extract and hyperforin DCHA with similar antidepressant-like effects also gave similar plasma levels of hyperforin (40-140 nM) (Cervo *et al.* 2002), further suggesting that hyperforin mainly accounts for these effects of the extracts. Laackmann *et al*. (1998) compared two different extracts with different hyperforin contents (0.5% WS5573 and 5% WS5572) in a randomized, double-blind, placebo-controlled, multicentre study. Only the high-hyperforin extract was superior to placebo in alleviating depressive symptoms in outpatients suffering from mild to moderate depression. However, in mice hyperforin at 4 and 8 mg/kg reduced immobility time but was inactive at lower and higher doses (Butterweck and Schmidt 2007). Hyperforin-free extracts still retain pharmacological activity in animal models (Butterweck *et al.* 2003), suggesting that other components might contribute to the antidepressant-like activity of SJW extracts in rodents.

Hyperforin may also have an important role in the effect of SJW extracts on ethanol intake (Perfumi *et al.* 2001; Wright *et al.* 2003). When tested in Marchigian Sardinian alcohol-preferring rats, a SJW extract enriched in hyperforin (24%) was 10 times more potent in reducing alcohol intake than the classic SJW extract (3.8% hyperforin) (Perfumi *et al.* 2001). Controversy exists on hyperforin's role in the anxiolytic-like effect of the extracts, possibly because of the different derivatives and experimental conditions used in the studies; hyperforin acetate (3-5 mg/kg) exerted poorly dose-related anxiolytic-like activity in the elevated plus-maze and in the light-dark test in rats (Zanoli *et al.* 2002), confirming earlier studies (Chatterjee *et al.* 1998b), whereas pure hyperforin (1-10 mg/kg) had no effects on stress-induced hyperthermia in mice (Grundmann *et al.* 2006). Therefore, further studies are needed to elucidate whether hyperforin has anxiolytic-like activity.

Rodent avoidance tests showed that hyperforin shares memory-enhancing properties with ethanolic SJW extracts, with hyperforin appearing a more potent anti-dementia agent than conventional antidepressants (Klusa *et al.* 2001). It has also been reported that hyperforin prevents the cognitive impairments observed in animal models of Alzheimer's disease (AD) (Griffith *et al.* 2010). Spatial memory impairments induced in rats by intrahippocampal injection of amyloid-β peptides ($A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$) were prevented by co-injection of 6 μM hyperforin-DCHA (Dinamarca *et al.* 2006). A different, possibly more important approach was then used to investigate the effects of tetrahydrohyperforin. The compound was administered chronically (2 mg/kg intraperitoneally once daily for four weeks) in transgenic (Tg) mice modeling an advanced stage of AD, i.e. already presenting Aβ plaques in their brain as well as cognitive impairments (Dinamarca *et al.* 2008; Cerpa *et al.* 2009). The results suggested a rescue of spatial memory in tetrahydrohyperforin-treated Tg mice.

Besides its central effects, SJW extracts are traditionally used for the topical treatment of superficial wounds, burns and dermatitis (Schempp *et al.* 2003) and hyperforin may have a key role. The finding of an inhibitory effect of hyperforin on the mixed epidermal cell lymphocyte reaction and on the proliferation of T lymphocytes provided a potential mechanism of action (Schempp *et al.* 2000). Hyperforin is also a potent inhibitor of cyclooxygenase-1 and 5-lipoxygenase, the key enzymes in the biosynthesis of proinflammatory leukotrienes (Albert *et al.* 2002; Feisst *et al.* 2009). Interest in hyperforin also increased in the light of its antibacterial and anti-tumoral activity (see for reviews (Beerhues 2006; Medina *et al.* 2006). including its possible inhibitory effects on tumour invasion and angiogenesis (Lorusso *et al.* 2009). Recent data also suggest that hyperforin might represent a new innovative therapeutic strategy in skin disorders characterized by altered keratinocyte differentiation (Müller *et al.* 2008).

However, as a matter of concern for the clinical use of SJW extracts, hyperforin is also likely to play a major role in the induction of the ATP-binding cassette transporter P-glycoprotein (Pgp) and of drug-metabolizing enzymes caused by SJW extracts (Mai *et al.* 2004; Mueller *et al.* 2006, 2009), sometimes with serious clinical consequences (reviewed by (Borrelli and Izzo 2009)). Indirect evidence comes from human studies showing that the extent by which different preparations of SJW extracts affect the pharmacokinetics of cyclosporin-A and midazolam (two substrates of CYP3A) correlates with their hyperforin content (Mai *et al.* 2004; Mueller *et al.* 2006). *In vitro* studies provided direct evidence of the inducing potential of hyperforin, showing that chronic treatment of human hepatocytes increased mRNA, protein, and the activity of CYP3A4 (Moore *et al.* 2000; Komoroski *et al.* 2004). This is possibly the consequence of the high-affinity binding (Ki=27 nM) of hyperforin with the pregnane X receptor (PXR) (Moore *et al.* 2000) a nuclear orphan receptor that controls metabolism and efflux transport of xenobiotics in barrier and excretory tissues (Wang and LeCluyse 2003; Bauer *et al.* 2004). *In vivo* studies confirmed these inducing properties, showing that repeated oral treatment of mice with hyperforin-DCHA actually increased the expression of hepatic CYP3A and the activity of CYP3A-dependent enzymes (Cantoni *et al.* 2003). Recently, Ott *et al.* (2009) provide first proof of principle that hyperforin, like other human but not rodent PXR ligands, activates pig PXR at the BBB and induce mRNA, protein expression, and transport activity of P-gp.

BIOAVAILABILITY AND BRAIN UPTAKE AND CONCENTRATIONS

In spite of the fact that hyperforin is one of the best–investigated components of SJW, possibly playing a significant role in most of the primary and secondary effects of the extracts, its molecular mechanism(s) of action is currently under investigation. Different hypotheses have been formulated on the basis of *in vitro*, *ex-vivo* and *in vivo* studies on neurochemical systems. However, in order to understand whether the interaction of a compound with a given neurochemical mechanism or receptor underlies the central effects of interest, one needs to know whether it actually reaches the systemic circulation and, more importantly, achieves high enough concentrations in the CNS after pharmacologically effective doses. For example, the affinity of quercetin and its main glycosides rutin and hyperoside for different rat neurotransmitter receptors was in the micromolar range in a recent *in vitro* receptor screening study (Butterveck *et al*. 2002). However, during and after absorption in man and animals flavonols are so extensively biotransformed that quercetin and its glycosides are generally detected in blood and tissues mainly as quercetin coniugates and *O*-methylated derivatives (Graefe *et al*. 2001). Only traces (about 0.1 nmol/g) of total quercetin and its *O*-methylated derivatives (i.e., quercetin and isorhamnetin aglycones after acid hydrolysis of the samples) were found in rat brain 4 h after high dose of a SJW extract containing about 100 mg flavonoids (Paulke *et al.* 2008). Although brain concentrations of total quercetin and isorhamnetin/tamarixetin rose slightly after daily doses of SJW extract in rats the results were not corrected for residual blood in the brain (Paulke *et al*. 2008), and therefore most of the observed brain concentrations may have been due to residual vascular content in brain tissue. Hence, the unchanged aglycone and its glycosides should not be able to interact *in vivo* with potentially relevant systems whose activation *in vitro* requires micromolar concentrations, and the *in vivo* effects of these flavonols are likely to be mostly due to their metabolites.

Table 1 Hyperforin steady-state plasma concentrations in man after daily doses of St. John's worth extracts, and brain availability in rat after pharmacologically effective doses of hyperforin derivatives.

Compound	Hyperforin concentrations	References
SJW Extract (mg/mg hyperforin)	***Human steady-state C_{max} (nmol/mL)***	
Sundow Herbals capsules (300/30, t.i.d.)	0.06 (0.03)	Hall *et al.* 2003
ST-3 tablets (612/13.5, once daily)	0.18 (0.06)	Schulz *et al.* 2005b
STW 3-VI tablets (900/17.2, once daily)	0.16 (0.07)	Schulz *et al.* 2005a
WS5572 tablets (900/42.8, once daily)	0.46 (0.04)	Biber *et al.* 1998
Hyperforin derivative (mg/kg)	***Rat brain concentrations (nmol/g)[a]***	
Hyperforin DCHA (0.19-0.38, i.p.)	<0.01	Cervo *et al.* 2002
Hyperforin DCHA (0.38, i.p)	1.6 (1%)	Mennini, personal communication
Hyperforin DCHA (12.5, i.p.)	0.06 (4%)	Cervo *et al.* 2002
Hyperforin TMB (0.5-0.7, i.p.)	<0.01	Cervo *et al.* 2005

DCHA = dicyclohexylammonium; TMB=trimethoxybenzoate . Both compounds were injected intraperitoneally three times in 24 h, and plasma and brain samples were obtained at the end of the behavioral test.

[a]The percentages of the corresponding plasma concentrations (assuming 1 g brain tissue equivalent to 1 mL water) are given in parentheses. Hyperforin brain levels were not corrected for residual blood in the brain

It follows that the neurochemical actions determined *in vitro* for hyperforin are meaningful only if compared with its plasma and brain concentrations after pharmacologically effective doses of pure compound or SJW extracts, like for other main components. The next section therefore focuses on the systemic bioavailability of hyperforin at the usual clinical doses of SJW extracts, and its relationship with brain availability which obviously is extrapolated from brain-to-blood distribution studies of authentic compound and extracts in animals.

According to the majority of clinical studies, the optimum dosage of SJW extracts for the treatment of mild depression is 900 mg/day (300 mg three times a day; t.i.d). Depending on the clinical response the dose can be increased to 1200 mg/day (Vitiello *et al.* 2005); comparable doses have been given on a once-daily basis in some studies (Biber *et al.* 1998; Schulz *et al.* 2005a, 2005b). Although the absolute oral bioavailability of this and other main components after these doses of commercial extracts is not known (Schulz *et al.* 2005a, 2005b) examined the pharmacokinetics of hyperforin, hypericin, pseudohypericin and total quercetin and total isorhamnetin after once-daily 612 mg STW-3 (Laif 600; 13.5 mg hyperforin) or 900 mg STW3-VI (Laif 900; 17.2 mg hyperforin); exposure to hyperforin was highest among the components considered (C_{max} of 87-97 μg/L, 0.16-0.18 μM, regardless of the extract); approximately 10-20 times higher than the naphthadianthrones and 3-10 times the total flavonols in terms of area under the plasma concentration-time curve (AUC), highlighting the potential role of hyperforin in the effects of the extracts. However, the content of SJW components may vary considerably between extracts, and consequently exposure to hyperforin may vary accordingly (see for review (Caccia 2005).

The disposition of hyperforin and other main components after oral SJW extracts have been reviewed (Caccia 2005; Caccia 2006; Wurglics and Schubert-Zsilavecz 2006). An important finding is that after single doses from 300 to 1200 mg of an alcohol/water extract (WS 5572, containing 5% hyperforin) in young male volunteers, the pharmacokinetics of hyperforin was almost proportional to the dose at 600 mg but less than proportional at 1200 mg (Biber *et al.* 1998). Mean elimination half-life ($t_{1/2}$) remained relatively constant across doses (8.5-9.7 h) but oral clearance progressively increased, reaching statistical significance at 1200 mg. After once-daily doses of 900 mg of WS 5572 (42.8 mg/day hyperforin) healthy subjects had mean C_{max} of 246 μg/L (0.46 μM). Accumulation was essentially complete within three days but exposure was about 30% lower than after the first dose (Biber *et al.* 1998). Similarly, after daily doses of 900 mg STW 3-IV extract (Laif 900; 17.2 mg hyperforin) the exposure to hyperforin was lower than after the first dose, although mean C_{max} (87.1 μg/L or 0.16 μM) (Schulz *et al.* 2005a) were lower than after WS 5572. This did not occur after daily supplementation for eight days with extracts containing only 4.5 mg hyperforin compared to the high hyperforin-extract (Biber *et al.* 1998) and was less marked with STW-3 (13.5 mg hyperforin) than with STW 3-IV (17.2 mg/day hyperforin), further suggesting that these changes are caused by hyperforin itself. Possibly there is an increase in hyperforin first-pass metabolism and clearance after repeated high-hyperforin extracts, as with many Pgp and CYP substrates given concomitantly with SJW (see above).

Hyperforin mean plasma C_{max} averaged 31 μg/L (0.06 μM) after 300 mg t.i.d Sundow Herbals capsules (containing about 30 mg of hyperforin) in healthy volunteers (Hall *et al.* 2003). Healthy subjects taking 300 mg t.i.d. Wild Oats capsules for 28 days (equivalent to about 12.2 mg of hyperforin) had mean hyperforin steady-state plasma concentrations (Css) of about 44 μg/L, ranging from <10 to 83 μg/L (0.15 μM) 1 h after the last dose (Cui *et al.* 2002). Hyperforin plasma C_{max} after higher doses t.i.d. have not been reported but plasma Css increased from 38.4 to 62 μg/L (0.07-0.12 μM) with the prescribed oral dosage of 900-1500 mg/day SJW in adult outpatients with major depression, within 8 hours of the last dose (Vitiello *et al.* 2005).

It appears that after the SJW doses used in humans, hyperforin may reach mean C_{max} around 100 nM after the t.i.d 300 mg schedule, with values slightly higher but less than expected after the once-daily high doses of 600-900 mg, i.e. 200-500 nM, with large inter-subject variability.

The scant data on the brain-to-blood distribution of hyperforin come from single-dose studies in rats. Hyperforin was not found in rat brain after pharmacologically equiactive intraperitoneal doses of hyperforin DCHA and hyperforin-rich SJW extract (0.19-0.38 and 3.12-6.25 mg/kg, respectively, given three times in 24 hours, i.e. the schedule of the forced swimming test (FST) (**Table 1**); these doses yielded plasma concentrations in the range of 40-120 nM (Cervo *et al.* 2002), comparable to those in man (see above). Hyperforin was measured in rat brain only after a much higher intraperitoneal dose of 12.5 mg/kg of the hyperforin salt, yielding plasma concentrations of about 1.4 μM. The brain concentrations were only about 60 nM (assuming 1 g brain tissue equivalent to 1 mL water) i.e. about 4% of the corresponding plasma levels, and possibly entirely related to hyperforin's contribution from blood (Cervo *et al.* 2002).

Another study evaluating hyperforin TMB in the FST model of depression (1.56–6.25 mg/kg, three intraperitoneal injections in 24 h) found hyperforin plasma concentrations from 30-70 nM but, again, brain levels were below the limit of quantification of the analytical procedure (Cervo *et al.* 2005). A more sensitive method was recently used to measure hyperforin concentrations in rat brain after an intraperitoneal dose of its DCHA salt effective in the FST (0.38 mg/kg) but these concentrations amounted to 0.85 ± 0.09 ng/g (1.6 nM), about 1% of the corresponding plasma levels (T. Mennini, pers. comm.).

These findings suggest that hyperforin crosses the BBB

poorly, with brain exposure (not corrected for residual blood) between 1-4% of the systemic exposure in rats. They also suggest that after the conventional doses of SJW extracts, the brain concentrations of hyperforin in man could be well below the 100-500 nM range found in blood, with values ≤ 20 nM (**Table 1**).

POTENTIAL MECHANISMS OF CENTRAL ACTIVITIES

As mentioned above, different hypotheses have been offered to explain the central effects of SJW extracts and hyperforin, mainly based on *in vitro* data. We briefly summarize these, in the light of the pharmacokinetic data described above.

Antidepressant-like and anxiolytic-like effects

The antidepressant properties of hyperforin have often been explained on the basis of its inhibition of the reuptake of monoamines at central synapses, through a mechanism of action similar to that of the most common antidepressants (Di Carlo *et al.* 2001). However, unlike conventional antidepressants, hyperforin is not a selective blocker of monoamine transporters, but its ability to inhibit neurotransmitter uptake – not only monoamines but also gamma-aminobutyric acid (GABA) and glutamate (Chatterjee *et al.* 1998a) (**Table 2**) – is the result of nonspecific interactions with all presynaptic terminals which are difficult to reconcile with the safety profile of SJW extracts (see also Gobbi and Mennini 2001). These 'presynaptic' effects occur *in vitro* at concentrations of 0.1–2 μM (Chatterjee *et al.* 1998a; Müller *et al.* 1998; Gobbi *et al.* 1999; Singer *et al.* 1999), i.e. higher than the brain concentrations found after effective doses of SJW or hyperforin (≤ 20 nM, see above). Much higher affinities (2–10 μM) were found testing hyperforin on most CNS receptors (**Table 2**) (Gobbi and Mennini 2005), including serotonin (5-HT) type 6 (5-HT_6) receptors (Kohen *et al.* 1996), 5-HT_7 receptors (Mnie-Filali *et al.* 2007), sigma1 receptors (Matsuno *et al.* 1996) and neuropeptide-Y receptors (Heilig 2004; Thorsell *et al.* 2006).

In vitro data seems to exclude that the anxiolytic-like effects are due to hyperforin interacting directly on the neurotransmitter potentially associated with increasing relaxation and reducing anxiety, such as GABA-A or benzodiazepine receptors (Gobbi *et al.* 2001) (Butterweck *et al.* 2002) or NPY receptors (Heilig 2004; Thorsell *et al.* 2006) (**Table 2**). Moreover, concentrations of hyperforin ≥ 0.5 μM were required to induce release of GABA from synaptosomes (Chatterjee *et al.* 2001).

Indirect effects can then be envisaged. For example, hyperforin might yield metabolites interacting with high affinity with the relevant receptors. The fact that *in vitro* it is a potent, competitive inhibitor of human CYP3A4 and CYP2C9 activities strongly suggests that it serves as a CYP substrate (Obach 2000). Accordingly, cytochrome P450-mediated oxidation may be a main route of hyperforin metabolism, based on preliminary *in vitro* studies in the rat (Komoroski *et al.* 2004), and oxidation of conventional antidepressants often results in active metabolites with cha-

Table 2 Affinity of hyperforin for different neurotransmitter receptors/transporters or for inhibition of neurotransmitter uptake, *in vitro*.

***In vitro* assay**	**Ki or IC_{50} (μM)**	**References**
synaptosomal 5-HT uptake	0.2-3.3	Chatterjee *et al.* 1998a; Gobbi *et al.* 1999; Singer *et al.* 1999
synaptosomal NA uptake	0.08	Chatterjee *et al.* 1998a
synaptosomal DA uptake	0.1-0.8	Chatterjee *et al.* 1998a; Gobbi *et al.* 1999
synaptosomal GABA uptake	0.18	Chatterjee *et al.* 1998a
synaptosomal GLUT uptake	0.83	Chatterjee *et al.* 1998a
5-HT transporters	> 18	Gobbi *et al.* 1999; Singer *et al.* 1999
DA transporters	2.6	Gobbi *et al.* 2001
NET transporters	> 10	Butterweck *et al.* 2002
5-HT1A receptors	> 10	Butterweck *et al.* 2002
5-HT1D receptors	> 10	Butterweck *et al.* 2002
5-HT1Dβ receptors	> 10	Butterweck *et al.* 2002
5-HT3 receptors	> 10	Butterweck *et al.* 2002
5-HT5a receptors	> 10	Butterweck *et al.* 2002
5-HT6 receptors	> 1-10	Gobbi *et al.* 2001; Simmen *et al.* 2001; Butterweck *et al.* 2002
5-HT7 receptors	> 1-10	Gobbi *et al.* 2001; Simmen *et al.* 2001; Butterweck *et al.* 2002
DA1 receptors	0.6	Butterweck *et al.* 2002
DA2 receptors	> 10	Butterweck *et al.* 2002
DA3 receptors	> 10	Butterweck *et al.* 2002
DA4 receptors	> 10	Butterweck *et al.* 2002
DA5 receptors	≥ 10	Butterweck *et al.* 2002
ACh-M1 receptors	> 10	Butterweck *et al.* 2002
ACh-M2 receptors	> 10	Butterweck *et al.* 2002
ACh-M3 receptors	> 10	Butterweck *et al.* 2002
ACh-M4 receptors	> 10	Butterweck *et al.* 2002
ACh-M5 receptors	> 10	Butterweck *et al.* 2002
GABA-A receptors	> 1-10	Gobbi *et al.* 2001; Butterweck *et al.* 2002
BDZ receptors	> 1-10	Gobbi *et al.* 2001; Butterweck *et al.* 2002
NPY-Y1 receptors	> 1	Gobbi *et al.* 2001
NPY-Y2 receptors	> 1	Gobbi *et al.* 2001
CRF1 receptors	>10	Simmen *et al.* 2001
SIGMA-1 receptors	> 1	Gobbi *et al.* 2001

racteristic pharmacokinetic and pharmacodynamic properties (Caccia 1998). It has also been suggested that the antidepressant-like effects of hyperforin in animal models indirectly involves sigma1 receptors (Cervo *et al.* 2005).

Like for other central effects, hyperforin might act synergistically or additively with other constituents, so that various relatively weak activities result in the overall pharmacological effect of the extracts. The biflavone amentoflavone, for example, bound to brain benzodiazepine receptors with an affinity comparable to diazepam, although injected in animals it does not bind these receptors possibly (Nielsen *et al*. 1988) because of its poor BBB penetration (Colovic *et al.* 2008). Amentoflavone, hypericin and miquelianin were active in the stress-induced hyperthermia model, which measures anti-stress or anxiolytic effects of psychoactive agents (Grundmann *et al.* 2006). Amentoflavone, its congener I3,II8-biapigenin, miquelianin and other quercetin glycosides (hyperoside, isoquercitrin and possibly rutin but not quercetin) are all active in tests predictive of antidepressant-like activity in animal models (Noldner and Schotz 2002; Butterweck *et al.* 2003). Hypericin and pseudohypericin too have antidepressant-like activity in behavioral tests in rodents although only at high doses or when given repeatedly (Butterweck *et al.* 2003). Therefore all these components are likely to contribute to the overall antidepressant/anxiolytic effects of SJW, although it remains to be established whether *directly* by interfering with certain neurotransmitter mechanisms or *indirectly* through the formation of as-yet unidentified metabolites.

It has been suggested (Gobbi *et al.* 2004) that hyperforin might exert its antidepressant effects by acting peripherally level (taking account of its negligible BBB passage), in particular by inhibiting inflammatory cytokine production and/or release (Dantzer *et al.* 2002). The hyperforin concentrations required *in vitro* to inhibit lipopolysaccharide-induced interleukin 6 (IL6) release from rat whole blood (1.3 μM) were not very different from the levels measured in plasma of rats treated with pharmacologically active doses of SJW (Biber *et al.* 1998; Cervo *et al.* 2002) or hyperforin DCHA (Cervo *et al.* 2002). The corresponding brain concentrations, as mentioned, are much lower with both.

Nootropic effects and antagonism of Aβ-induced cognitive impairments

Different mechanism(s) have been suggested to explain the nootropic effects detected with hyperforin and SJW extracts in rodents (Khalifa 2001; Klusa *et al.* 2001; Widy-Tyszkiewicz *et al.* 2002; Trofimiuk and Braszko 2008). Besides the possibility of changes in the brain levels of some neurotransmitters (Widy-Tyszkiewicz *et al.* 2002), interesting data showed that hyperforin activates TRPC6 channels (Leuner *et al.* 2007; Tu *et al.* 2009), thus possibly favoring the formation of excitatory synapses and enhancing spatial learning and memory (Zhou *et al.* 2008). The *direct* effect of hyperforin on TRPC6 channels has only been assessed using quite a high concentration (10 μM). The same authors, however, reported that much lower – and pharmacologically relevant – hyperforin concentrations (0.1 μM) induced neurite outgrowth in PC12 cells in a TRPC6-dependent manner (Leuner *et al.* 2007).

The beneficial effects with hyperforin and its reduced derivative tetrahydrohyperforin on the cognitive impairments induced by Aβ (Dinamarca *et al.* 2006, 2008; Cerpa *et al.* 2009) were associated with a direct interaction with Aβ *in vitro*: disaggregation of Aβ fibrils induced by 46 nM hyperforin (Dinamarca *et al.* 2006) or release of acetylcholinesterase from Aβ plaques induced by 150 nM tetrahydrohyperforin (Dinamarca *et al.* 2008). Nanomolar concentrations of hyperforin-DCHA (10-100 nM) were enough to prevent the neuronal death induced by Aβ oligomers (Dinamarca *et al.* 2006) which are now thought to be the most toxic Aβ species (Haass and Selkoe 2007; Shankar *et al.* 2007; Selkoe 2008).

CONCLUDING REMARKS

The main conclusion that can be drawn from the *in vitro*, *ex vivo* and *in vivo* studies of hyperforin so far is that its role in the therapeutic potential of SJW extracts for psychiatric illnesses (mainly depression, but also anxiety) are unlikely to be due to a *direct* interaction with acknowledged neurotransmitter transporters and receptors. This is mainly because the concentrations required for these interactions exceed those reached in rodent brain by this poorly CNS-available component after pharmacologically effective doses of SJW or authentic compound (≤ 20 nM); the same applies in man, considering the hyperforin steady-state concentrations after conventional doses of the extracts, and assuming that the component brain-to-blood partition is similar to that in rats (see **Table 1**). Further pharmacological studies are now required to clarify hyperforin's role in the antidepressant/anxiolytic-like effects of the extracts, and the mechanism(s) of action.

As regards the nootropic and anti-Alzheimer effects of hyperforin, different hypotheses involve either a direct effect on Aβ or a neurotrophic effect. Hyperforin concentrations as low as 10-100 nM inhibited the toxic effects of Aβ oligomers and induced neurite outgrowth. Again, further studies are required to confirm and extend these promising findings.

Considering that hyperforin is possibly a CYP substrate eliminated by CYP-mediated oxidation, and that oxidized metabolites of conventional psychotropic drugs may have characteristic pharmacokinetic and pharmacodynamic properties, formal metabolic studies in man and animals are essential. Research should also provide additional information on the brain uptake of concentrations of hyperforin compared to its active metabolite, to enable us to identify the active principle (parent compound or its metabolite) and its target in the brain, and will undoubtedly help in extrapolating pharmacological findings across species. These studies should obviously be extended to all the main components of SJW extracts; previous *in vitro* findings have often been over-interpreted, mainly because they have been considered without taking into account the (scant) pharmacokinetic and metabolism data available for all components.

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