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Role of Hyperforin in the Pharmacological Activities of St. John's Wort

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ABSTRACT

The phloroglucinol derivative hyperforin has been recently shown to be a major antidepressant component in the extract of *Hypericum perforatum*. Experimental studies clearly demonstrated its activity in different behavioral models of depression. Moreover clinical studies linked the therapeutic efficacy of *Hypericum* extracts to their hyperforin content, in a dose-dependent manner.

The molecular mechanism of action of hyperforin is still under investigation. Hyperforin has been shown to inhibit, like conventional antidepressants, the neuronal uptake of serotonin, norepinephrine and dopamine. However, hyperforin inhibits also the uptake of γ-aminobutyric acid (GABA) and L-glutamate. The uptake inhibition by hyperforin does not involve specific binding sites at the transporter molecules; its mechanism of action seems to be related to sodium conductive pathways, leading to an elevation in intracellular Na^+ concentration. Other additional mechanisms of action of hyperforin, involving ionic conductances as well synaptosomal and vesicular function, have been suggested. In addition to its antidepressant activity, hyperforin has many other pharmacological effects *in vivo* (anxiolytic-like, cognition-enhancing effects) and *in vitro* (antioxidant, anticyclooxygenase-1, and anticarcinogenic effects). These effects could be of clinical importance. On the other hand, the role of hyperforin in the pharmacological interactions occurring during *Hypericum* extract therapy must be fully investigated. Hyperforin seems to be responsible for the induction of liver cytochrome oxidase enzymes and intestinal P-glycoprotein.

Several pharmacokinetic studies performed in rats and humans demonstrated oral bioavailability of hyperforin from *Hypericum* extract. Only recently a new chromatographic method for detection of hyperforin in the brain tissue has been developed and validated. Taking into account the chemical instability of hyperforin, current efforts are directed to the synthesis of new neuroactive derivatives.

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INTRODUCTION

Hypericum perforatum, commonly known as St. John's wort (SJW), is a medicinal plant widely used in Europe and in the USA as an alternative treatment for mild to moderate forms of depression. It gained widespread popularity as "natural Prozac," and its efficacy has been confirmed by many but not all clinical studies (8,35). However, SJW is not indicated in the treatment of severe depression (89).

Several bioactive compounds have been identified in the commercially available extract of the plant: naphthodianthrones (hypericin, pseudohypericin), phenylpropanes (e.g., chlorogenic and caffeic acids), flavonol glycosides (e.g., quercetin), biflavones (biapigenin), proanthocyanidins (e.g., procyanidin), phloroglucinols (hyperforin, adhyperforin) (9,67,68). Xanthones have been reported to be present in the roots of *Hypericum perforatum*, although they are not normally present in the commercial extracts.

In spite of the fact that *Hypericum perforatum* is one of the best-investigated medicinal plants, it is still a matter of debate which component accounts, wholly or partially, for the antidepressant activity. Different studies have ascribed the antidepressant property of *Hypericum* extracts to the naphthodianthrones hypericin and pseudohypericin (16,18), to flavonoids (17,22) and to the phloroglucinol derivative hyperforin (25,50,65,66,92,106). The role and the mechanisms of action of these different compounds are still under investigation. In particular, increasing interest has been focused on hyperforin, the most abundant lipophilic component of *Hypericum* extract.

This article reviews published studies on the pharmacological activity of hyperforin and its possible role in the therapeutic effects of *Hypericum* extract.

CHEMISTRY

Hyperforin is the major of the two acylphloroglucinols present in *Hypericum perforatum*. It was discovered in 1971 as an antibacterial principle in St. John's wort by Gurevich et al. (47).

The structure and the physicochemical characteristics of hyperforin were described in numerous publications starting in 1975 (12,13,21). The basic structure of hyperforin was elucidated primarily on the basis of its chemical degradation. The attempts to confirm the proposed structure of hyperforin by complete synthesis have failed up to now; but recently a model system for the synthesis of phloroglucinol containing natural products has been developed (56). The isolation and identification of a hyperforin homolog, adhyperforin, a minor acylphloroglucinol-type component from the aerial parts of *Hypericum perforatum*, has been reported (60).

Hyperforin is present in an amount of approximately 5% of the dry weight in the flowers and leaves of *Hypericum perforatum*, but, due to its chemical instability, the content of hyperforin in improperly dried products might decrease drastically. Analysis carried out by the high-performance liquid chromatographic method on commercially dried extracts revealed that hyperforin content in the extracts can range from 1.18 to 6.54% (4). The temperature and light sensitivities of pure hyperforin, as well as of *Hypericum* commercial products, have been extensively studied (7,70).

Hyperforin is a bicyclic compound of meroterpenoid origin (Fig. 1). Its biosynthesis involves five isoprenoid moieties, predominantly derived from the deoxyxylulose phosphate pathway. Using quantitative NMR spectroscopy Adam (1) analyzed hyperforin isolated

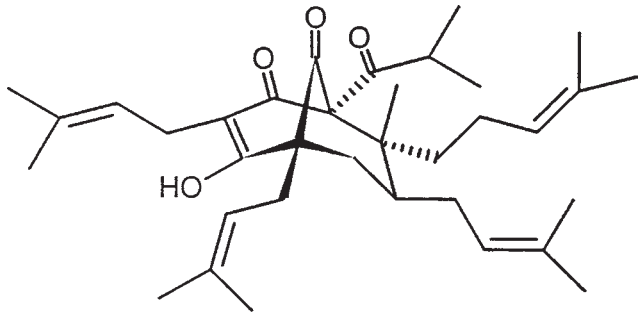

Fig. 1. Structure of hyperforin.

from cut sprouts of *Hypericum perforatum* that was immersed in a solution containing [1–^{13}C]glucose or [U–$^{13}C_6$]glucose.

Hyperforin has pronounced susceptibility to oxidative transformation; it is highly sensitive to heat and light, either in powder or in solution (70). In the crude ethanolic extract of the aerial parts of *Hypericum perforatum*, two oxidized products of hyperforin have been identified by Trifunović et al. (94). Subsequently, the presence of furohyperforin, named also orthoforin, was reported by Verotta et al. (97) and by Orth et al. (71). Three additional oxygenated hyperforin analogs were isolated by Verotta (98). Further degradation products, such as pyranohyperforin, and more recently deoxyfurohyperforin A have been identified in the aerial parts of the plant (87,96).

PHARMACOLOGY

Behavioral Studies

Several pharmacological studies demonstrated the antidepressant activity of *Hypericum perforatum* extract in experimental animals (5,15,34,42). Preliminary studies with either hyperforin or hyperforin-enriched CO_2 extract (5,25) suggested that the phloroglucinol derivative exerts antidepressant activity. The dominant role of hyperforin was demonstrated by several authors in different experimental rodent models of depression: forced swimming test, tail suspension test, learned helplessness test or acute-escape deficit induced by an unavoidable stress (20,24,26,43,108). In these studies pure hyperforin or more stable salts, such as dicyclohexylammonium (DCHA), acetate or sodium hyperforin, were used (28,99). The above-mentioned susceptibility of the natural molecule to oxidative degradation might affect its pharmacological activity.

An inverse U-shaped dose-response curve for hyperforin acetate has been observed in rats subjected to forced swimming test: the antidepressant-like activity was observed with hyperforin acetate only at doses ranging from 5 to 20 mg/kg (108). This finding was recently confirmed in mice subjected to the tail suspension test: pure hyperforin, at doses from 4 to 8 mg/kg, significantly reduced immobility time, while it was inactive at either lower or higher doses (20). In this study the authors demonstrated, however, that SJW extract free of hyperforin and hypericin but enriched in flavonoids, exerted antidepressant activity, confirming the hypothesis that several components of SJW extract with a different mechanism of action may be responsible for the therapeutic efficacy of the plant (17,20).

The anti-immobility effect of hyperforin, observed in different tests of antidepressant activity, may not be related to the unspecific locomotor stimulation, which was excluded by us and others (24,108). On the contrary, Buchholzer et al. (14) observed a decrease in locomotor activity in mice treated with 10 mg/kg of hyperforin sodium salt. The same dose of hyperforin acetate reduced the number of crossed areas in the open field test in rats (108).

In addition to the well-described antidepressant activity, other behavioral effects of hyperforin have been described by us and others (14,24,26,54,108). Of particular interest is the anxiolytic activity, elicited in rats by 3–5 mg/kg of hyperforin acetate in the elevated plus maze test. This effect was inhibited by pretreatment of animals with metergoline, a serotoninergic antagonist, but not with 2-phenylpyrazolo[3,4-c]quinolin-3(5H)-one (CGS-8216), a benzodiazepine receptor antagonist (108). These results excluded the involvement of benzodiazepine receptor system, as confirmed by *in vitro* experiments (45), but suggested participation of a serotonergic mechanism in hyperforin activity. An anxiolytic-like effect of *Hypericum* extract has been previously suggested (5,95).

It must be stressed that the anxiolytic-like effect of hyperforin was observed after a single administration, while the antidepressant activity was observed only after repeated doses (26,108). It could mean that the mechanisms responsible for the two effects of hyperforin are quite different.

Hyperforin sodium salt, administered subacutely to rats (1.25 mg/kg/day) or acutely to mice (1.25 mg/kg), improved memory acquisition and consolidation in the conditioned and passive avoidance tests (54). It appears that hyperforin enhances cognition at doses lower than it is effective in the forced swimming test, but has a similar inverted U-shaped dose-response curve. Moreover, hyperforin almost completely reversed scopolamine-induced amnesia in mice (54).

Since depressive disorders and alcohol abuse may imply similar neurochemical changes in the central nervous system, such as a hypofunction of the serotoninergic system, it has been hypothesized that SJW extract could effectively suppress alcohol intake. Studies performed in different genetic animal models of human alcoholism demonstrated the efficacy of *Hypericum* extract in reducing ethanol intake and suggested its potential therapeutic use in the treatment of alcoholism (73,74,78,107). Since CO_2 extract (enriched in hyperforin) was more potent than methanolic extract, it has been speculated that hyperforin has the primary role in reducing alcohol intake by *Hypericum* extract (74,107).

Molecular Mechanism of Action

The majority of antidepressant drugs lead to an increased synaptic availability of norepinephrine and serotonin, as a consequence of monoamine oxidase (MAO) inhibition or of monoamine reuptake inhibition. This last biochemical mechanism is shared by almost all old and new antidepressants.

A clear inhibitory effect on the synaptosomal uptake of monoamines was previously demonstrated for SJW extract (25). The same investigators found that, while neither hypericine nor flavonoids had any reuptake inhibiting property, hyperforin, at a low micromolar range, was a potent synaptosomal uptake inhibitor for 5-HT, DA, NE, and GABA with almost equal potencies (IC_{50} values ranged from 0.04 to 0.10 μg/mL) (25). On the other hand, hyperforin did not appear to inhibit MAO, since hyperforin-enriched CO_2 extract, in comparison to methanolic extract, had only very weak MAO-A and MAO-B inhibitory properties (65). Hyperforin is, however, not the only component of SJW with a

potent inhibitory effect on synaptosomal uptake of monoamine neurotransmitters: its close derivative adhyperforin, present in the extracts at ten times lower levels than hyperforin, has the same inhibitory profile and is as potent as hyperforin (50,106). Furohyperforin, a polar analog of hyperforin, was, however, only 1/10 as potent as hyperforin in 5-HT synaptosomal uptake studies. Taking into account the low concentration of furohyperforin in the extract (~5% of hyperforin), one can exclude a significant neuroactive role for furohyperforin (97).

As observed by Chatterjee (25), hyperforin inhibits GABA and L-glutamate uptake systems, with rather similar IC_{50} values in a high nanomolar range (143 and 184 nM, respectively), close to its IC_{50} values for 5-HT, DA, NE uptake (105). No other antidepressant compound exhibits a similar broad uptake inhibitory profile. Thus, according to the currently predominant opinion, the inhibition of monoamines uptake by hyperforin is not due to a selective blockade of neurotransmitter transporters (44,45,66,92), but more likely due to a non-specific effect on synaptosomal ionic homeostasis (27,57,92). The neurotransmitter transporters are characterized by direct coupling of substrate to an inward cotransport of Na^+ ions, which provides the driving force for the accumulation of substrate in the cell (61). Hyperforin slightly increases free intracellular Na^+ and, therefore, impairs Na^+-dependent transporters. It has been inferred that this effect can lead to a decreased neurotransmitter uptake (92). The increase in $[Na^+]_i$ was observed in rat brain synaptosomes and also in human platelets at the hyperforin concentrations capable of inhibiting serotonin uptake. The maximal effect was seen at 5 μM of hyperforin. At higher concentrations hyperforin had no further effects or was significantly less active (66). Comparing the effects of hyperforin to those of sodium ionophore, monensin, it has been speculated that hyperforin is not a simple sodium ionophore, but affects Na^+–H^+ exchanger. A possible role of amiloride sensitive sodium conductive pathways has been suggested, since at certain concentrations benzamil (inhibitor of amiloride sensitive Na^+-channels) and 5′-ethylisopropylamiloride (EIPA) (inhibitor of Na^+–H^+ exchanger) reduced hyperforin effect on L-glutamate uptake (66,105). However the experiments failed to suggest the specific mechanism involved in the inhibition of monoamine uptake by hyperforin.

The assumption that hyperforin non-selectively activates sodium channels is supported by electrophysiological findings indicating that hyperforin modulates several ionic conductance mechanisms, including Na^+, K^+, and Ca^{2+} voltage-dependent channels in rat cerebellar Purkinje cells. In addition, inhibitory effects of hyperforin on ligand-operated ion channels of AMPA, NMDA, and GABA receptors have been observed (27,39). It must be emphasized that the inhibitory effects of hyperforin on voltage- and ligand-gated ion channels have been observed at low micromolar concentrations, whereas its effects on monoamine transporters occur at nanomolar range (65,81).

Apparent non-specific uptake inhibition can also be induced by compounds that affect storage vesicles and raise the cytoplasmic levels of neurotransmitters, an effect similar to that of reserpine at the monoaminergic systems. Gobbi et al. (44) showed that hyperforin has a reserpine-like mechanism *in vitro*, since it inhibited [^{3}H]5-HT accumulation in rat brain synaptosomes and increased cytoplasmic concentration of 5-HT, suggesting an impairment of monoamine storage in the vesicles. Since hyperforin also induces the release of some amino acids from synaptosomes it is possible that, unlike reserpine, hyperforin affects storage vesicles non-specifically, by modulating intracellular ion concentrations. Such an effect would explain the inhibition of synaptosomal accumulation of amino acids (14,25,29,44). This effect is preceded in rat cortical synaptosomes by an increase in free

calcium levels. Since this event is observed in cerebral cortical synaptosomes, as well as in a smooth muscle cell line, in the absence of Ca^{2+} in the medium, the authors speculated that the rise in $[Ca^{2+}]_i$ is not due to increased Ca^{2+} entry, but more likely due to a release of Ca^{2+} from intrasynaptosomal storage sites or due to a reduction in the Ca^{2+} buffering capacity (29,55). It is, however, well known that synaptic neurotransmitter release (and/or uptake) is not only a calcium-dependent process, but is also regulated by different interdependent mechanisms.

The involvement of sodium and calcium ions in hyperforin activity has been recently confirmed by Marsh (62). In mouse cortical brain slices perfused by hyperforin (5 μM), a consistent enhancement in amino acids (glutamate, aspartate, serine, glycine, and GABA) levels has been observed. This effect was inhibited by perfusion with a sodium channel blocker, tetrodotoxin (TTX, 1 μM), and potentiated by removal of extracellular calcium (62).

Similarly to the synaptosomal uptake, the vesicular uptake of 5-HT, NE, and DA is non-competitively inhibited by hyperforin (80). Moreover, the concentrations of hyperforin capable of inhibiting the vesicular monoamine uptake are almost identical to those needed for inhibition of the synaptosomal uptake of the same monoamines. The inhibition does not seem to involve direct recognition of the vesicular transporter by hyperforin. A possible explanation could be an interference with the driving force of the vesicular uptake. The pH gradient across the synaptic vesicle membrane, induced by H^+-ATPase, is the major driving force for vesicular monoamines uptake and storage (93). Hyperforin has been demonstrated to reverse a generated pH gradient in vesicles (29) and has been recently described to dissipate an existing pH gradient across synaptic vesicle membrane (81).

In order to clarify the molecular mechanism of action of hyperforin, binding studies related to various central nervous system receptors have been carried out (19,45,91). These studies did not identify any receptor to which hyperforin binds with sufficiently high affinity, i.e., at lower than micromolar range, that could account for its antidepressant activity (45). It should be pointed out that at the therapeutic doses of *Hypericum* extract (300 mg containing ~5% hyperforin) the maximal plasma levels of the drug have been reported to reach approximately 280 nM and that the steady-state plasma levels in humans after repeated doses of the extract (300 mg/day × 3 days) could reach approximately 180 nM (6). Therefore, the clinically observed antidepressant activity of SJW extract, that depends on hyperforin content, is most probably due to its effects on uptake systems rather than on the neurotransmitter receptor-mediated responses.

An extensive study on the effects of hyperforin at various receptors was carried out by Butterweck et al. (19). On all tested receptors hyperforin was less potent than the naphtodianthrone hypericin and the biflavonoid amentoflavone. Hyperforin significantly inhibited cloned D_1 and D_5 dopamine receptors and human cloned norepinephrine transporter. This last finding favored the previously suggested mechanism for the inhibition of synaptosomal monoamine reuptake (65,106). The relative affinity for dopaminergic receptors could also be of interest taking into account the involvement of the dopaminergic system in depression and the therapeutic effects of antidepressants, such as nomifensine, bupropion and others.

Since the common biochemical marker of antidepressant activity is downregulation of central β-adrenergic receptors, this effect was investigated with SJW extracts and its components. While chronic treatment with *Hypericum* extract downregulates β_1-adrenoceptors

(65,90), short-term (2 weeks) or long-term (8 weeks) treatments with hyperforin failed to affect the density of β-adrenoceptors (90).

The influence of hyperforin on neuronal excitability was investigated in guinea pig hippocampal slices by Langosch et al. (59). While commercially available *Hypericum* extract produced concentration-dependent excitatory effects, hyperforin was found to be slightly excitatory, and only at 1 μM, a much higher concentration than can be reached with the commercial extract. Thus, hyperforin seems not likely to be responsible for the excitatory effects of *Hypericum* extract. At higher concentrations (10–100 μM) hyperforin seems to inhibit synaptic transmission, since it reduces population spike amplitude.

The *in vivo* studies performed with hyperforin in animals (Table 2), generally confirmed the results obtained *in vitro* (Table 1). By systemic administration, at 10 mg/kg, i.p., hyperforin enhanced the extracellular concentrations of monoamines and glutamate in the locus coeruleus of anesthetized rats (52,77) and caused a significant elevation of striatal acetylcholine release (14). On the other hand, the levels of the amino acids GABA, taurine, serine, arginine in the rat locus coeruleus, were not modified by intraperitoneal injection of hyperforin (52,77).

Other Pharmacological Effects

The antibacterial property of hyperforin has been known for a long time (47). Since it was reported to inhibit multidrug resistant *Staphylococcus aureus*, hyperforin attracted renewed interest as an antibacterial agent (83).

An antioxidant activity of hyperforin as well as other phloroglucinol derivatives was demonstrated in different cellular and enzymatic assays (48). In addition, hyperforin has been found to act as a dual inhibitor of 5-lipoxygenase and cyclooxygenase-1, key enzymes in the production of proinflammatory eicosanoids from arachidonic acid, suggesting its therapeutic potential in inflammatory diseases (2).

The ability of hyperforin to affect intracellular pH regulation prompted the investigation, carried out by Froestl *in vitro*, on the effect of hyperforin on the processing of amyloid precursor protein (APP) (40). The authors showed that hyperforin activates APP secretory processing, probably through a direct effect on α-secretase. Since this proteolytic enzyme is associated with the cell membrane, it cannot be excluded that the effect of hyperforin on membrane fluidity (37) or its cyclooxygenase-1 and 5-lipoxygenase inhibitory properties (2) may be relevant for its effect on APP processing.

Interesting results concerning the anticarcinogenic property of hyperforin have been reported. Hyperforin has been shown to exert an inhibitory effect on human epidermal cells and on the proliferation of phytohemagglutinin-stimulated peripheral blood mononuclear cells (84). In addition, hyperforin has been reported to have a cytostatic activity in autologous MT-450 breast carcinoma of rats (85). The antiproliferative effect of hyperforin has been demonstrated in human malignant cell lines and correlated to the induction of apoptosis involving a caspase-dependent pathway (49).

PHARMACOKINETICS

It is well known that hyperforin is highly lipophilic, temperature sensitive, susceptible to photodegradation and decomposes quickly in non-polar reagents, such as hexane (70). Therefore, analytical methodologies should strive to avoid conditions that may adversely

affect the stability of hyperforin. Few high-performance liquid chromatography (HPLC) methods have been reported for the determination of hyperforin in human plasma.

A liquid-liquid extraction using hexane-ethyl acetate and HPLC analysis with tandem mass spectrometry (MS-MS) detection was reported by Biber et al. (6). This method was highly sensitive: the lower limit of detection was 1 ng/mL. In healthy volunteers, after an oral dose of 300 mg *Hypericum* extract containing 14.8 mg hyperforin, the maximum plasma levels (150 ng/mL) were reached at 3.5 h after administration; its half-life was 9 h. In a repeated dose study (3 × 300 mg/day of the extract), the estimated steady state plasma concentration of hyperforin, was approximately 100 ng/mL. There was no accumulation of hyperforin in plasma.

Since the method described by Biber (6) was expensive for routine measurements, Chi and Franklin (31) developed a rapid and cheap procedure utilizing solid-phase extraction (SPE) and HPLC separation with ultraviolet (UV) detection. It must be noticed that the assay range was 150–300 ng/mL and the extraction recovery tests for hyperforin were performed at concentrations >300 ng/mL, values exceeding those found in humans after administration of SJW.

In the HPLC-UV method described by Bauer (3), the use of hexane-ethyl acetate in liquid-liquid extraction might potentially affect hyperforin stability.

More recently a simple and reproducible HPLC method, utilizing a solid-phase extraction, was described by Cui (32). High absolute recovery values were obtained by this method without the use of non-polar solvents and inorganic acids, which might impair hyperforin stability. Hyperforin was detected in human plasma after ingestion of a single 900 mg dose of a commercially available SJW extract containing 8.55 mg hyperforin. The maximal plasma concentration of hyperforin was 27.6 ng/mL, the time to maximal concentration 4.4 h and the elimination half-life 3.5 h. Hyperforin plasma levels have been also measured in subjects after repeated administration (3 times daily for 28 days) of the SJW extract containing the daily dose of approximately 12 mg hyperforin. The mean plasma levels for each subject ranged from <10 to 82.78 ng/mL.

After systemic administration of hyperforin sodium salt, 10 mg/kg, to rats, plasma levels of hyperforin were in the micromolar range (14). With a lower dose of hyperforin, the drug was not detectable in the plasma of rats.

There is little information available on the passage of hyperforin through the blood-brain barrier, brain uptake or its brain levels. An attempt to measure brain levels of hyperforin after administration of SJW extract or hyperforin DCHA was reported by Cervo et al. (24), but hyperforin brain levels were below the limit of detection by the analytical procedure used in this study.

In another study [^{14}C]hyperforin was isolated by an elaborate procedure from the plant after its *in situ* synthesis. After administration of the labeled molecule, radioactivity was detected in the brain (72).

Only recently a new technique of high-performance liquid chromatography/tandem mass spectrometry was developed and used to determine hyperforin levels in the murine brain after oral administration of hyperforin sodium salt (15 mg/kg) or SJW extract (containing 5% hyperforin, 300 mg/kg). The mean brain level of hyperforin was 28.8 ng/g in the animals receiving sodium salt, while the brain levels were lower in the extract-treated group. This highly sensitive and selective method allows detection of very low brain levels of hyperforin, ranging from 2.5 to 100 ng/g of brain tissue (53).

The metabolism of hyperforin in the liver was studied *in vitro* using rat liver microsomes. The isoforms CYP3A and CYP2B of the cytochrome P450 appear to be respon-

sible for the hydroxylation reactions leading to the production of hyperforin phase I metabolites (19-hydroxyhyperforin, 24-hydroxyhyperforin, 29-hydroxyhyperforin, and 34-hydroxyhyperforin) (33).

PHARMACOLOGICAL INTERACTIONS

Recently great interest has been raised over interactions between SJW extract and some important drugs such as cyclosporine, HIV protease inhibitors, cytostatic compounds, anticoagulants, oral antidiabetics or contraceptives (38,41,82). The finding of decreased plasma levels of these drugs and the clinical consequence of their reduced efficacy is today an important issue for *Hypericum* extract.

Several *in vitro* and clinical studies provided evidence that SJW enhances metabolic degradation of drugs by inducing cytochrome P450 (CYP) drug metabolizing enzymes in the liver (36,51,63,64,79). In particular, the expression of both hepatic and intestinal CYP3A4 appeared to be induced through the action of hyperforin on the pregnane X receptor system, which regulates the expression of cytochrome CYP3A4 monooxygenase (64,103). The high affinity of hyperforin as a ligand for pregnane X receptor seems to be responsible for the induction of CYP2C9 catalytic activity detected in primary human hepatocytes (30,103). An *in vitro* study showed, however, that hyperforin is a competitive inhibitor of CYP3A4 and a non-competitive inhibitor of CYP2D6 (69). Moreover, hyperforin has been shown to inhibit CYP1A2, in addition to CYP2C9 and CYP2C19 (69,109). These discrepancies in the results of *in vitro* experiments (enzyme induction or inhibition) could be due to different sources, enzyme substrates or methods of analysis.

An increase in intestinal P-glycoprotein (P-gp), responsible for active transport of drugs across membrane bilayers, was assessed *in vivo* as a consequence of SJW chronic use (36) and *in vitro* after exposure to SJW or hypericin (75). However, hyperforin as well as hypericin can initially inhibit effective function of P-gp-mediated efflux, as observed *in vitro* by Wang (102).

In vivo experiments demonstrated that short-term treatment (4 days) with SJW (435 mg/kg) as well with pure hyperforin (10 mg/kg) failed to induce CYP1A2, CYP2E1, and CYP3A isoforms in the male Swiss Webster mice (10). There was no change in total hepatic CYP450 or in the catalytic activity or polypeptide levels of the three isoforms of CYP450. The influence of hyperforin on the liver drug metabolizing system was investigated in rats injected with a single dose of pentobarbital after acute and chronic (4 and 7 days) treatments with hyperforin (5–10 mg/kg) (108). A lack of an effect on hepatic enzymes was suggested by the observation that, in both cases, hyperforin failed to alter pentobarbital sleeping time. On the other hand, a recent study in mice, treated with SJW (300 mg/kg) or hyperforin (18.1 mg/kg), both for 4 and 12 days, suggested that hyperforin behaves qualitatively and quantitatively like the extract in inducing CYP3A4 activity (23). Since these results contradict those reported above, further studies are needed to assess the conditions required for the alteration of CYP450 activity by hyperforin.

CLINICAL STUDIES

Several clinical studies with SJW extracts provided evidence of therapeutic efficacy of the extracts in mild depression. The efficacy of the extracts was similar to that of many conventional antidepressant agents, but their side effect profile was more favorable (11,76, 86,101,104).

TABLE 1. Summary of relevant reports on "in vitro" activity of hyperforin

Investigator (reference)	Tissue	Assay	Effect of hyperforin
Chatterjee et al. (25)	Mouse and rat brain synaptosomal preparation	Synaptosomal uptake of DA, NE, 5-HT, GABA, L-glutamate	Biogenic amines and amino acids uptake inhibition (IC_{50} from 0.043 to 0.445 μg/mL)
	Mouse brain homogenate	MAO assays	No inhibition of MAO-A and MAO-B activities
Chatterjee et al. (27)	Rat hippocampal neurons	Voltage- and ligand-gated ionic conductance	Induction of a dose and time dependent inward current GABA, AMPA and NMDA conductance inhibition
Gobbi et al. (44)	Rat brain synaptosomes	Synaptosomal 5-HT and DA uptake	5-HT and DA uptake inhibition (IC_{50} = 1.8 μg/mL and IC_{50} = 0.44 μg/mL, respectively)
Wonnemann et al. (105)	Mouse brain synaptosomes	Amino acids synaptosomal uptake	Inhibition of GABA and L-glutamate uptake, counteracted by amiloride derivatives
Chatterjee et al. (29)	Rat brain synaptosomes	Amino acids release	Stimulation of glutamate, aspartate and GABA release
		Synaptosomal $[Ca^{2+}]_i$ and pH assay	Increase in $[Ca^{2+}]_i$
Gobbi et al. (45)	Rat brain membranes	Binding assay of 5-HT_6, 5-HT_7, sigma, GABA/BZD, NPY receptors and DA transporters	DA transporter inhibition (IC_{50} = 2.6 μg/mL)
Butterweck et al. (19)	Human cloned receptors	Binding assays of GPCRs and neurotransmitter transporters	Inhibition of hD_1- and hD_5-dopamine receptors
			Inhibition of NE transporter
Buchholzer et al. (14)	Rat brain synaptosomes	Synaptosomal choline uptake	Inhibition of high-affinity choline uptake (IC_{50} = 8.5 μM)
Marsh and Davies (62)	Mouse brain slices	Release of amino acid neurotransmitters	Increased release of amino acids after perfusion with 1–5 μM of hyperforin; effect inhibited by sodium channel blocker (TTX)
Langosch et al. (59)	Guinea pig hippocampal slices	Extracellular electrophysiology	Spike amplitude increase (1 μM) and decrease (10–100 μM)
Roz et al. (81)	Rat brain synaptosomes	Presynaptic monoamine uptake	Inhibition of monoamine presynatic uptake
		Vesicular monoamine uptake	Reduced vesicular storage of monoamines
		ATP-dependent proton uptake	Reversal of generated pH gradient

TABLE 2. Summary of relevant reports on "in vivo" activity of hyperforin

Investigator (reference)	Animal	Hyperforin	Dose	Treatment	Test	Effect of hyperforin
Chatterjee et al. (26)	rat	Pure	20 mg/kg/day p.o.	3 days	Behavioral despair	Reduction in immobility time
	rat		0.3–3 mg/kg p.o.	acute	Elevated plus maze	Increase in % entries and time spent in open arms
Kaehler et al. (52)	rat	Pure	10 mg/kg i.p.	acute	Push-pull superfusion of locus coeruleus	Increased extracellular brain concentrations of 5-HT, NE, DA, and L-glutamate
Gambarana et al. (43)	rat	Pure	12.5–75 mg/kg i.p.	acute	Escape deficit	Increased number of escapes after exposure to unavoidable stress
Buchholzer et al. (14)	rat	Sodium	1–10 mg/kg i.p.	Acute	Microdialysis	Increase of striatal ACh release
	mouse		1–10 mg/kg i.p.	acute	Locomotor activity	Decreased locomotor activity (10 mg/kg)
Cervo et al. (24)	rat	Dicyclohexylammonium	0.10–0.38 mg/kg i.p.	3 times in 24 h	Behavioral despair	Reduction in immobility time (0.19–0.38 mg/kg)
					Open field	No change in locomotor activity
Zanoli et al. (108)	rat	Acetate	3–40 mg/kg p.o.	3 times in 24 h	Behavioral despair	Reduction in immobility time (5–20 mg/kg)
			5–10 mg/kg p.o.	7 days	Learned helplessness	Increased escape responses (10 mg/kg)
			1–10 mg/kg p.o.	acute	Elevated plus maze	Increased time spent in open arms (3–5 mg/kg)
			1–10 mg/kg p.o.	acute	Open field	Reduced number of crossed areas (3–10 mg/kg)
Butterweck et al. (20)	mouse	Pure	2–20 mg/kg p.o.	acute	Tail suspension test	Reduction in immobility time (4–8 mg/kg)
Simbrey et al. (90)	rat	Trimethoxybenzoate	8 mg/kg/day p.o.	2 weeks	β-adrenergic receptor binding assay	No effect in both treatment paradigms
			8 mg/kg/day p.o.	8 weeks		

The clinical antidepressant efficacy of standardized extracts has been correlated, in a dose-dependent manner, with their hyperforin content (46,58). The effects of SJW extracts with a high content of hyperforin (5%) have been compared in human volunteers using quantitative topographic EEG with the effects of extracts containing only 0.5% of hyperforin and found to have a more pronounced effect on the central nervous system (88). No clinical studies have been performed with the pure hyperforin.

SUMMARY

The phloroglucinol derivative hyperforin is the main lipophilic chemical component of *Hypericum perforatum* extract. Experimental evidence suggests that hyperforin exerts a clear antidepressant effect. The antidepressant activity was confirmed by clinical studies with *Hypericum perforatum* extracts. These studies established a direct correlation between the therapeutic efficacy of SJW extracts and their hyperforin content.

In comparison with all other antidepressants hyperforin possesses a unique pharmacological profile, because it inhibits the uptake of 5-HT, norepinephrine, dopamine as well as glutamate and GABA. This action is not associated with a specific binding to different transporter molecules, but with a mechanism involving Na^+ conductive pathways that is relevant for the activity of all neurotransmitter transporters. Moreover, hyperforin has been found to: *a*) directly stimulate the release of neurotransmitters from synaptosomes; *b*) modulate several ligand- and voltage-dependent ion channels conductances; and *c*) affect synaptosomal and vesicular pH. In spite of these relevant findings, further studies are needed to clarify the mechanism(s) of action of hyperforin.

In addition to its antidepressant activity, hyperforin elicits, in experimental animals, an anxiolytic effect. This effect could be clinically important in view of the documented efficacy of selective serotonin uptake inhibitors (SSRIs) in anxiety disorders.

The observed cognition-enhancing activity of hyperforin in rodents suggests a new and interesting therapeutic perspective for this drug in the treatment of depressive disorders associated with cognitive disturbances. The beneficial effect of hyperforin in learning and memory tests could be explained by facilitation of release of acetylcholine in the brain.

Finally, hyperforin could be considered useful in the treatment of malignant disorders since it has antiproliferative and apoptosis-inducing activities.

The findings described above may have future therapeutic implications but require validation by further experimental and clinical studies. The activity, efficacy and safety of hyperforin will have to be better defined and particular attention should be given to the effects of hyperforin on the drug metabolizing enzymes and its potential interaction with other drugs.

The pharmacokinetic studies demonstrated that hyperforin is an orally bioavailable component of *Hypericum* extract. With the adequate dosing schedule its steady state concentrations in human plasma can be easily achieved and maintained.

The chemical instability is of crucial importance for the quality of commercial products. Consequently, the current promising research on new synthetic neuroactive derivatives of hyperforin is fully justified (99–100).

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