LEADING ARTICLE

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Mechanism of Action of St John's Wort in Depression

What is Known?

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Abstract

Extracts of *Hypericum perforatum* L. (St John's wort) are now successfully competing for status as a standard antidepressant therapy. Because of this, great effort has been devoted to identifying the active antidepressant compounds in the extract. From a phytochemical point of view, St John's wort is one of the best-investigated medicinal plants. A series of bioactive compounds has been detected in the crude material, namely flavonol derivatives, biflavones, proanthocyanidines, xanthones, phloroglucinols and naphthodianthrones.

Although St John's wort has been subjected to extensive scientific studies in the last decade, there are still many open questions about its pharmacology and mechanism of action. Initial biochemical studies reported that St John's wort is only a weak inhibitor of monoamine oxidase-A and -B activity but that it inhibits the synaptosomal uptake of serotonin, dopamine and noradrenaline (norepinephrine) with approximately equal affinity. However, other *in vitro* binding assays carried out using St John's wort extract demonstrated significant affinity for adenosine, $GABA_A$, $GABA_B$ and glutamate receptors. *In vivo* St John's wort extract leads to a downregulation of β-adrenergic receptors and an upregulation of serotonin 5-HT_2 receptors in the rat frontal cortex and causes changes in neurotransmitter concentrations in brain areas that are implicated in depression. In studies using the rat forced swimming test, an animal model of depression, St John's wort extracts induced a significant reduction of immobility. In other experimental models of depression, including acute and chronic forms of escape deficit induced by stressors, St John's wort extract was shown to protect rats from the consequences of unavoidable stress. Recent neuroendocrine studies suggest that St John's wort is involved in the regulation of genes that control hypothalamic-pituitary-adrenal axis function. With regard to the antidepressant effects of St John's wort extract, many of the pharmacological activities appear to be attributable to the naphthodianthrone hypericin, the phloroglucinol derivative hyperforin and several flavonoids.

This review integrates new findings of possible mechanisms that may underlie the antidepressant action of St John's wort and its active constituents with a large body of existing literature.

1. St John's Wort as an Antidepressant

Mental illness imposes a tremendous burden on the Western world. Mental disorders can strike early in life, and they are increasing in incidence in an aging population experiencing neurodegenerative diseases. The search for new and more effective therapeutic agents includes the study of plants used in traditional medicine systems to treat mental disorders. In the last 10–15 years, phytomedicines based on extracts from the herb of *Hypericum perforatum* L. (St John's wort, Clusiaceae) have gained widespread popularity as "the Prozac of herbs".[1]

Over the past 2000 years, St John's wort has been singled out for its diverse medicinal properties by eminent medical writers and folk healers; the primary ancient medical herbalists, including Hippocrates, Pliny, Dioscurides and Galen, wrote about the medicinal properties of St John's wort, noting its use as a wound-healing, diuretic and antimalarial agent. It was also used in a number of European countries for the treatment of neuralgia, menopausal neurosis, anxiety and depression and as a nerve tonic (for review see Bombardelli and Morazzoni[2]). Today, alcoholic extracts produced from the upper third of the flowering plant are used for the treatment of mild to moderate depression. Preparations of St John's wort are some of the most prescribed medicines in Germany; the number of prescriptions for them have approximately tripled since 1993 (in 1999 approximately 130 million daily doses were prescribed).[3,4] In the US, St John's wort is increasingly used as an over-the-counter remedy for the treatment of depression.[5]

1.1 Evidence of Efficacy

The antidepressant efficacy of St John's wort extracts has been confirmed in several clinical studies.[6-11] A meta-analysis that was published in 1996 by Linde et al.[12] of trials of St John's wort concluded that there is good evidence that St John's wort is superior to placebo in treating mild to moderate depression. However, the authors emphasised the need for more studies with larger samples and particularly for trials comparing St John's wort with conventional antidepressants.

Since the meta-analysis appeared,[12] several more double-blind, randomised, controlled trials have been published that have indicated the efficacy of St John's wort in depression.[9,11,13-15] However, a recent large-scale negative study by Shelton et al.[16] raised concerns about the effectiveness of the herb. It should be emphasised that in this clinical trial, patients with severe depression were included, an indication for which St John's wort is not approved in any country (despite an initial trial that suggested efficacy in this population[14]). A large-scale study coordinated by researchers of the National Institutes of Mental Health (NIMH) in the US tried to address some of the unanswered questions.[17] St John's wort was compared with placebo and an SSRI (sertraline) in 340 patients for 8 weeks initially and a further 4 months for those who responded positively. The study failed to support the efficacy of St John's wort in severe depression. While the authors found no evidence for a superior effect of St John's wort relative to placebo, sertraline could also not be differentiated from placebo on the primary efficacy measures, which casts doubt on the validity of the study. Whether the doses used in this trial were sufficiently high for treating severe depression is questionable, since the response rates from both groups were relatively low. Thus, the results of the trials[16,17] involving severely depressed patients are unconvincing, and further studies are required to investigate the effectiveness of St John's wort in comparison with conventional antidepressants in patients with severe depression.

Comparisons of different St John's wort preparations and dose-finding studies are still lacking. The trial by Laakmann et al.[15] is the only investigation comparing two different extracts of St John's wort. It showed that an extract with 5% hyperforin was significantly more effective, in terms of reducing the Hamilton Depression Rating Scale score, than an extract with 0.5% hyperforin. This finding suggested that hyperforin may be of relevance for the clinical efficacy of the extract. However, data on the other constituents (such as flavonoids, hypericins and biflavones) in the extracts were not presented

and, thus, it cannot be excluded that constituents other than hyperforin may have clinical efficacy.[18]

It can be concluded that several clinical trials strengthen the case for St John's wort as an antidepressant, but more work needs to be done to answer the remaining questions. In particular, the identification of the effects of different (analytically well characterised) preparations, administration schedules, optimal doses and treatment durations would be valuable.

2. Constituents

From a phytochemical point of view, St John's wort is one of the best-investigated medicinal plants. A series of bioactive compounds has been detected in the crude material, namely phenylpropanes, flavonol derivatives, biflavones, proanthocyanidines, xanthones, phloroglucinols, some amino acids, naphthodianthrones and essential oil constituents (for reviews see Bombardelli and Morazzoni,[2] Nahrstedt and Butterweck[19] and Nahrstedt[18]) [structures are shown in figure 1 and figure 2].

Although St John's wort has been subjected to extensive scientific studies in the last decade, there are still many open questions about its pharmacology and mechanism of action. In fact, the active constituents are not fully known. Recently, the antidepressant activity of St John's wort extracts has been variously attributed to the phloroglucinol derivative hyperforin,[22-25] to the naphthodianthrones hypericin and pseudohypericin[20,26-28] and to several flavonoids.[29,30] The role and mechanisms of these different compounds are still a matter of debate. However, based on recent results, it seems that the prevailing simplistic view of one plant → one active compound → one mechanism of action is incorrect. It is more likely that the multiple bioactive compounds contribute to the antidepressant activity of the crude plant extract in a complex manner.

This review focuses on the present knowledge about the mechanism of action of St John's wort and its active constituents in depression, with regard to the most recent literature.

3. St John's Wort and the Common Hypotheses of Antidepressant Action

3.1 Inhibition of Monoamine Oxidase

Several early *in vitro* experiments with St John's wort focused on pathways that alter monoamine neurotransmission in the CNS. Initial reports suggested that inhibition of monoamine oxidase (MAO) – the enzyme that is responsible for the catabolism of biogenic amines – is the main mechanism of antidepressant action of St John's wort extract. Based on the work of Suzuki et al.,[31] hypericin was considered to be an inhibitor of both MAO type A and type B. Demisch et al.[32] and Sparenberg et al.[33] confirmed the inhibitory effect on MAO of the St John's wort extract but not of hypericin. The authors found MAO inhibitory effects by the flavonoids and xanthones.

Bladt and Wagner[34] investigated the effect on MAO of six fractions from St John's wort extract. Inhibition of MAO-A could be shown with the total extract at a relatively high concentration of 10^{-3} mol/L (based on a molecular weight [MW] of quercitrin of 449). In 1994, Thiede and Walper[35] investigated the effects of St John's wort extract, hypericin and some St John's wort fractions on the activity of MAO and catechol-*O*-methyltransferase (COMT). Inhibition of MAO was observed with St John's wort extract 10^{-4} mol/L (based on an average MW of 500) and hypericin 10^{-3} mol/L. Only the St John's wort extract inhibited COMT. The isolated fractions were less active in both enzyme assays. The authors concluded that the clinically proven antidepressant activity of St John's wort cannot be explained in terms of MAO inhibition. These findings were later supported by Cott[36] and Müller and coworkers.[37] Both authors confirmed the rather weak potency of St John's wort extract as an inhibitor of MAO-A and -B.

Suzuki et al.[31] were the first to find that micromolar concentrations of hypericin could irreversibly inhibit MAO-A and -B activity *in vitro*. However, the progress made in preparation and analysis techniques since 1984 has shown that the hypericin used in these experiments was impure and

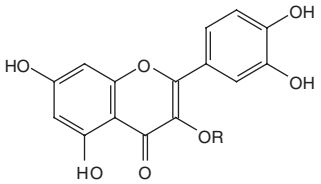
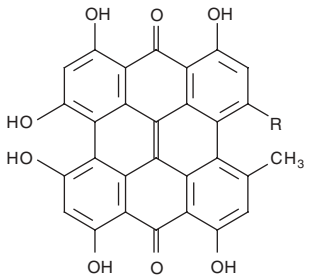
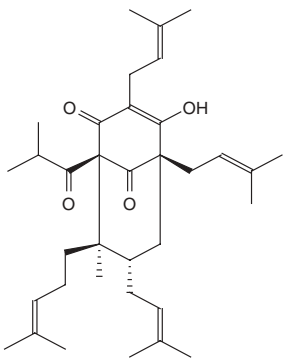
Group of bioactive compound	Structure	Constituent		Approximate % amount in a crude St John's wort extract*
Flavonoids		1. R = H 2. R = α-L-rhamnosyl 3. R = β-D-glucosyl 4. R = β-D-galactosyl 5. R = β-D-rutinosyl 6. R = β-D-glucuronide	Quercetin Quercitrine Isoquercitrin Hyperoside Rutin Miquelianin	1. 0.2–0.6 2. 0.2–0.4 3. 0.5–0.8 4. 2–4 5. 2–3 6. 0.4–0.05
Naphthodianthrones		1. R = CH_3 2. R = CH_2OH	Hypericin Pseudohypericin	1. 0.08–0.20 2. 0.15–0.40
Phloroglucinols		Hyperforin		0.9–5

Fig. 1. Chemical structures of the major constituents of St John's wort: flavonoids, naphthodianthrones and phloroglucinols. * Data on content of bioactive compounds in crude extract from Butterweck et al.,[20] Nahrstedt and Butterweck[19] and Jürgenliemk and Nahrstedt.[21]

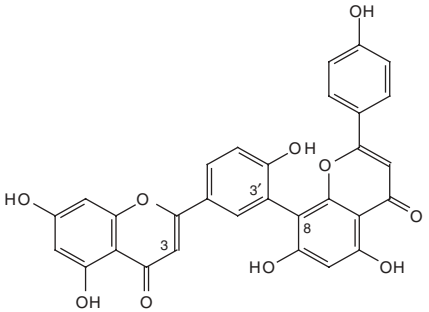
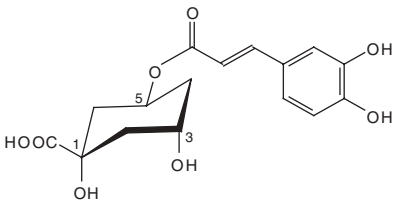
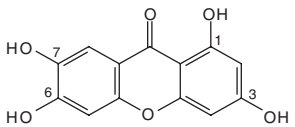
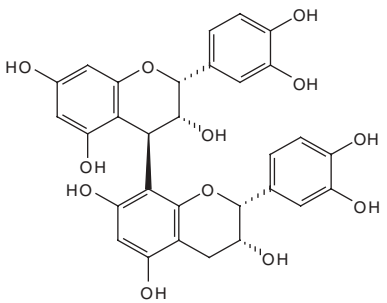
Group of bioactive compound	Structure	Constituent	Approximate % amount in a crude St John's wort extract*
Biflavones		1. I3, II8-biapigenin 2. I3′, II8-biapigenin = amentoflavone	1. 0.2–0.6 2. 0.01–0.05
Phenylpropanes		Chlorogenic acid	<1
Xanthones		1,3,6,7-Tetrahydroxyxanthone	<0.0004
Proanthocyanidines		Procyanidin B2	Total amount of proanthocyanidines including B2 ~8.8

Fig. 2. Chemical structures of the major constituents of St John's wort: biflavones, phenylpropanes, xanthones and proanthocyanidins. * Data on content of bioactive compounds in crude extract from Butterweck et al.,[20] Nahrstedt and Butterweck[19] and Jürgenliemk and Nahrstedt.[21]

contained at least 20% of other constituents of the extracts; among these, the flavonoids are the most noticeable. In fact, the MAO inhibitory effects of hypericin could not be confirmed in subsequent studies.[32,34-36] It was suggested that the flavonoid derivatives, therefore, might be responsible for the inhibitory effects on MAO, as the chemical structure of those compounds is similar to that of synthetic MAO inhibitors.[38] Indeed, the flavonoids quercetin, luteolin and kaempferol have been shown to inhibit MAO activity *in vitro*.[35] However, the concentration in St John's wort of these substances, especially that of the aglycones, is too low to be responsible for the therapeutic efficacy of St John's wort extracts.[18,21] Chatterjee et al.[22] investigated the phloroglucinol derivative hyperforin and found that it did not inhibit MAO-A or -B activities.

Data from all the above-mentioned studies are summarised in table I. In conclusion, although several studies demonstrate that MAO inhibitors are present in St John's wort, their relatively high inhibitory concentrations do not suggest any relevance of MAO-A or -B inhibition to the antidepressant effect of St John's wort. These results suggest that other mechanisms of action contribute to the antidepressant activity of the herb.

3.2 Inhibition of Synaptosomal Reuptake of Amines

Reuptake into nerve terminals is the main mechanism for terminating the actions of synaptically released biogenic amines such as serotonin, noradrenaline (norepinephrine) and dopamine.[40-42] Inhibition of the reuptake, via blockade of distinct transporters, is a common action of a variety of antidepressants, including tricyclic antidepressants (TCAs) and SSRIs,[43,44] and results in an increase of monoamine activity at synapses.[45,46]

Research with St John's wort also focused on inhibition of synaptosomal reuptake of serotonin and/or noradrenaline; it was assumed that this might point to an initial mechanism of action of the plant extract similar to that of most other antidepressant drugs. All data regarding monoamine reuptake are summarised in table II.

The first studies in this field were performed by Perovic and Müller.[50] The authors showed that St John's wort extract is a rather potent inhibitor of synaptosomal serotonin reuptake. This finding was confirmed by Müller and Schäfer,[39] who found that St John's wort extract inhibited synaptosomal reuptake of serotonin, noradrenaline and dopamine with quite similar potencies.

In order to identify the major reuptake-inhibiting compound of St John's wort, initial research focused

Table I. Monoamine oxidase (MAO) inhibition by St John's wort extract and various compounds

References	MAO-A and -B inhibition (μmol/L range)
Hydroalcoholic St John's wort extract	
Bladt & Wagner,[34] Chatterjee et al.,[22] Cott,[36] Demisch et al.,[32] Müller and Schäfer,[39] Müller et al.,[37] Sparenberg et al.,[33] Suzuki et al.,[31] Thiede & Walper[35]	+
Hypericin	
Suzuki et al.[31]	+
Bladt & Wagner,[34] Cott,[36] Demisch et al.,[32] Sparenberg et al.[33]	−
Hyperforin	
Chatterjee et al.[22]	−
Quercitrin	
Bladt & Wagner,[34] Demisch et al.,[32] Sparenberg et al.,[33] Thiede & Walper[35]	+
Quercetin	
Demisch et al.,[32] Sparenberg et al.,[33] Thiede & Walper[35]	+
1,3,6,7-Tetrahydroxyxanthone	
Demisch et al.,[32] Sparenberg et al.,[33] Suzuki et al.[31]	+

+ indicates inhibition; − indicates no effect.

on the lipophilic phloroglucinol derivative hyperforin. Later systematic studies included a broad spectrum of compounds. The first studies with hyperforin were performed by Chatterjee et al.[22] and Müller and colleagues.[48] The authors found that hyperforin was capable of inhibiting the reuptake of all three monoamines with a potency comparable to that of conventional serotonin and noradrenaline reuptake inhibitors. Moreover, the authors noted that the ability of hyperforin to inhibit the reuptake of serotonin, noradrenaline and dopamine at a nanomolar concentration sets it apart from any known synthetic antidepressant. Subsequent papers confirmed that hyperforin is a monoamine reuptake inhibitor, but only at micromolar concentrations.[47,49,52] In these studies, hypericin was inactive in all monoamine systems. This finding was also confirmed by Kientsch et al.[51] and Raffa.[53]

In a recent study, Wonnemann et al.[25] systematically investigated the synaptosomal reuptake inhibitory properties of several constituents of St John's wort. The authors again identified hyperforin as the major reuptake-inhibiting principle; however, it has to be mentioned that this result was not obtained using the pure compound but rather by correlation analysis of IC_{50} values (concentrations that produce 50% inhibition of reuptake) of ten different St John's wort extracts based on different amounts of hyperforin. Further, analytical data of additional constituents in the extracts, such as flavonoids or biflavones, are missing. Thus, it cannot be excluded that constituents other than hyperforin may have caused the synaptosomal reuptake inhibition.

Interestingly, in the same study, the authors[25] found that hyperforin-free preparations were also able to inhibit the reuptake systems in a weak to moderate manner. A fraction containing mostly oligomeric procyanidins showed a weak to moderate inhibition of synaptosomal reuptake of serotonin, noradrenaline, dopamine, GABA and L-glutamate. Although these data are interesting, the important information of specific activity is missing: procyanidins are tannins and as such they precipitate proteins.[54] However, the nonspecific inhibition of transport proteins by procyanidins is not excluded by the results of this study.

Table II. Comparison of the effects of different St John's wort extracts, pure compounds and standard synthetic antidepressants on reuptake of monoamines[a]

Substance	Serotonin reuptake	Noradrenaline (norepinephrine) reuptake	Dopamine reuptake
St John's wort extracts			
Hydroalcoholic St John's wort extract	+[22,37,39,47-50]	+[22,37,39,48,49,51]	+[22,37,39,48]
CO_2 extract	+[48,52]	+[47,48]	+[47,48]
Hyperforin[b]	+[22,25,47-49,52]; –[51]	+[22,25,48,49,52]; –[51]	+[22,25,48,49,52]
Adhyperforin	+[25,52]	+[25,52]	+[25,52]
Hypericin	–[50,51,53]	–[48,51,53]	–[48,51,53]
Hyperoside	–[25]	–[25]	–[25]
Biapigenin	–[25]	–[25]	–[25]
Quercitrin	–[25]	–[25]	–[25]
Isoquercitrin	–[25]	–[25]	–[25]
Rutin	–[25]	–[25]	–[25]
Amentoflavone	–[25]	–[25]	–[25]
Oligomeric procyanidins	+[25]	+[25]	+[25]
Standard antidepressants			
Imipramine	+[37,52]	+[37,52]	–[37,52]
Nomifensine	–[37,52]	+[37,52]	+[37,52]
Fluoxetine	+[52]	+[52]	–[52]

a This table presents an overview of investigations into effects on monoamine uptake, but it does not consider quantitative differences because the original data from different authors had a different 'cut-off' for activity. Activities were observed in a nmol/L to μmol/L range.

b In the study by Wonnemann et al.,[25] various extracts with different amounts of hyperforin were tested; pure hyperforin was not tested.

+ indicates inhibition of uptake; – indicates no inhibition of uptake.

Recently, Kientsch et al.[51] investigated the acute actions of the St John's wort extract ZE 117 on neurotransmitter reuptake into rat brain cortex slices. The ZE 117 extract (standardised to 0.2% hypericin, <0.1% hyperforin) was more selective for the reuptake of noradrenaline than for serotonin. Interestingly, none of these effects could be achieved with either hypericin or hyperforin in a relevant dose range. The authors concluded that "the extract ZE 117 contains active, but as yet unknown antidepressant principles with effects comparable to

those of TCAs". Systematic studies confirming this hypothesis are required.

Buchholzer et al.[55] presented the first data on interactions of hyperforin with the cholinergic system. The authors found that hyperforin inhibited high-affinity choline reuptake in rat synaptosomes. These results are of special interest and could form the basis for the investigation of the use of hyperforin in cognitive disorders.

3.3 Effects on Monoamine Transporters

In order to explain the mechanism of inhibition of synaptosomal reuptake, several researchers have focused on the effects of St John's wort on monoamine transporters in intact neural cells. The neurotransmitter transporters have been proven to be important targets for CNS drug discovery, particularly for antidepressants.[40]

Gobbi et al.[47] reported that inhibition of serotonin reuptake by a methanolic St John's wort extract and hyperforin is not due to a direct interaction with, and blockade of, the serotonin transporter because both compounds had no or only a very slight inhibitory effect on [^{3}H]citalopram binding. The authors speculated that the inhibitory effects on synaptosomal serotonin reuptake might be due to a reserpine-like mechanism, because similar results were obtained with Ro-04-1284, a reserpine-like compound, that also did not inhibit [^{3}H]citalopram binding. Their hypothesis was confirmed by the finding that St John's wort extract and hyperforin induced a marked release of serotonin from synaptosomes preloaded with [^{3}H]serotonin. The authors concluded that the apparent inhibition of reuptake is an artefact due to the interaction of the high concentration of the tested compounds with monoamine storage vesicles.

Jensen et al.[52] found that the potency of hyperforin and adhyperforin in terms of inhibition of monoamine reuptake was comparable to that seen with imipramine, nomifensine and fluoxetine. But in contrast to the antidepressant drugs, the phloroglucinols potently inhibited all three transporter systems. Interestingly, the inhibition of dopamine uptake by hyperforin and adhyperforin was not due to a direct interaction with the [^{3}H]WIN 35,428 (a cocaine analogue) binding site on the dopamine transporter as seen for imipramine, nomifensine and fluoxetine. Therefore, the authors suggest a noncompetitive interaction of hyperforin and adhyperforin with the dopamine transporter.

Another interesting hypothesis regarding the mechanism of synaptosomal reuptake is presented by Müller et al.[24] and Singer et al.[23] The apparent inhibition of serotonin reuptake observed with St John's wort extract and hyperforin *in vitro* might be due to an increase in free intracellular sodium concentrations.[23] Additional data suggest interactions of hyperforin with Na^+ channels or Na^+/H^+ exchangers.[56] Such nonselective effects might explain why St John's wort extracts and hyperforin blocked the synaptosomal reuptake of multiple neurotransmitters.[22,23,47,56]

Recent work indicated that hyperforin may also influence calmodulin-dependent mechanisms.[57] Hyperforin was also reported to modulate several ionic conductances in cerebellar Purkinje cells including a P-type calcium channel that is known to be involved in neurotransmitter release.[57] Krishtal et al.[58] also screened an ethanolic St John's wort extract (hyperforin content approximately 5%) on various voltage- and ligand-gated conductances. The plant extract inhibited almost all the ligand-gated ion channels; quercitrin was detected as a potent inhibitor of adenosine triphosphate (ATP)-induced conductance; biapigenin inhibited both the acetylcholine- and ATP-induced conductance, whereas hyperoside blocked currents activated by ATP and α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA). Hypericin was inactive in all cases. The authors concluded that hyperforin is not the only active substance of St John's wort and that the extract contains several potentially neuroactive molecules, such as biapigenin, hyperoside and quercitrin.

The concentration of hyperforin (micromolar range) used in these studies[57-59] was far higher than plasma concentrations reached *in vivo*. In human volunteers receiving daily doses of St John's wort extract 900mg (three 300mg doses), steady-state

plasma concentrations of approximately 180 nmol/L have been measured.[60] Reported IC_{50} values for hyperforin as an inhibitor of synaptosomal reuptake of serotonin have ranged from 120 to 3300 nmol/L. According to the results of the studies mentioned above,[23,24,47,52] the blood concentrations of hyperforin in human volunteers after a daily dose of St John's wort extract 900mg are within a concentration range needed to inhibit serotonin reuptake *in vitro*, but the free drug concentration available for action at central ion channels *in vivo* is unclear.

Taken together, the *in vitro* effects of St John's wort and hyperforin clearly show that both are potent but nonspecific inhibitors of synaptosomal serotonin, noradrenaline and dopamine, but that the precise mechanism relevant to therapeutic actions is still unknown. It is likely that other mechanisms may also contribute to the overall antidepressant action of St John's wort. Because all these data were obtained in *in vitro* experiments, it is of interest to know if these interactions with monoamine systems actually occur *in vivo*.

3.4 Effects on Brain Monoamine Levels

3.4.1 Single-Dose Treatment

Calapai et al.[30] demonstrated that a methanolic extract of St John's wort (125–500 mg/kg orally), like fluoxetine, increased serotonin levels in the cortex and noradrenaline and dopamine levels in the diencephalon after a single dose in rats. Similar findings were reported by Yu,[61] who observed an increase of serotonin in mouse hippocampal and hypothalamic tissues after a single dose of a St John's wort extract (extract not further characterised; 10–100 mg/kg orally). However, this author noted that there were no significant changes of noradrenaline, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) or homovanillic acid (HVA) levels. The author also reported unchanged caudate and cortex serotonin levels.

Serdarevic et al.[62] determined neurotransmitter levels in whole mouse brains after single-dose treatment with a methanolic St John's wort extract (250–500 mg/kg orally). In this study, serotonin levels remained unchanged, whereas levels of the serotonin metabolite 5-hydroxyindolacetic acid (5-HIAA) were significantly increased after treatment with 500 mg/kg of the plant extract; no effects on noradrenaline levels were observed, and DOPAC and HVA levels were significantly increased.

In the study by Fornal et al.,[63] two commercial preparations of St John's wort (Jarsin®300[1], Hyperforat®) failed to affect neuronal activity in the dorsal raphe nucleus of awake cats after single-dose treatment, whereas fluoxetine and sertraline markedly decreased neuronal activity by increasing the synaptic availability of serotonin at inhibitory somatodendritic $5\text{-}HT_{1A}$ autoreceptors.

Di Matteo et al.[64] used *in vivo* microdialysis to investigate the effects of a CO_2 extract (not further characterised) on dopamine, DOPAC, serotonin and 5-HIAA levels. At a dose of 1 mg/kg orally, the extract caused a slight but significant increase of dopamine outflow both in the nucleus accumbens and the striatum. No significant changes in serotonin or 5-HIAA efflux were observed. Similar results were reported by Rommelspacher and colleagues:[65] after single-dose administration of a methanolic St John's wort extract (31.25–125 mg/kg orally) or a CO_2 extract (2.42–9.68 mg/kg orally), dopamine and serotonin levels were increased in the nucleus accumbens.

Franklin et al.[66] measured monoamine levels in whole brain tissues after a single intraperitoneal dose of a methanolic St John's wort extract (50–100 mg/kg), hypericin (0.4 mg/kg) or hyperforin (9.3 mg/kg). While the extract (100 mg/kg) increased levels of dopamine and serotonin, hypericin and hyperforin had no effect on monoamine levels.

Using the microdialysis technique, Kaehler et al.[67] and Philippu[68] found enhanced extracellular levels of dopamine, noradrenaline, serotonin and glutamate in the rat locus coeruleus after a single intraperitoneal injection of hyperforin 10 mg/kg. Levels of 5-HIAA, GABA, taurine, aspartate, serine and arginine were not influenced. In the same study, a hyperforin-free St John's wort extract also elevat-

1 The use of tradenames is for product identification purposes only and does not imply endorsement.

ed extracellular levels of catecholamines. In contrast to hyperforin, the extracellular level of serotonin was decreased. Therefore, the author[68] concluded that the differing profiles of hyperforin and hyperforin-free St John's wort extract on extracellular transmitter levels point to the presence of an additional biologically active compound in St John's wort.

Based on the results in the above-mentioned studies, the effects of single doses of St John's wort extract on monoamine levels seem to be relatively inconsistent. These inconsistencies could be explained by: (i) anatomically different brain regions assessed; (ii) different techniques (microdialysis, brain tissues, electrophysiological recordings); (iii) differences in the phytochemical composition of the extracts (variation of plant material, methanolic or CO_2 extract); (iv) different administration routes (orally, intraperitoneally, intravenously); and (v) different extract doses.

3.4.2 Longer Term Treatment

Despite the importance of data from single-dose treatment studies, the therapeutic mode of action of St John's wort needs to be established in studies based on repeated treatment. Data after repeated treatment are of special interest because of the classically observed time lag between onset of antidepressant drug therapy and reversal of the depressed mood. Like imipramine, the onset of efficacy of St John's wort extract occurs typically after 2–3 weeks of treatment, so it is believed that the medication causes delayed CNS adaptations.[69,70]

In a recent study, Rommelspacher et al.[65] showed that after 14 days of daily administration, a methanolic St John's wort extract (31.25–125 mg/kg orally) had no effects on dopamine levels in the rat nucleus accumbens, whereas a CO_2 extract (2.42–9.68 mg/kg orally) caused a clear increase. Interestingly, after the same treatment period, both extracts significantly increased serotonin levels in this brain region.

Butterweck et al.[71] recently demonstrated that a St John's wort extract and the naphthodianthrone hypericin affected levels of monoamine neurotransmitters and their metabolites after long-term (8 weeks) administration, similar to imipramine (15 mg/kg orally). All three drugs (St John's wort extract, hypericin and imipramine) increased serotonin levels in the hypothalamus after long-term (8 weeks) but not short-term (2 weeks) administration. Interestingly, serotonin levels remained unchanged in hippocampal tissues after both treatment paradigms. The level of noradrenaline was not changed after short-term (2 weeks) treatment with either imipramine or St John's wort extract, but it was reduced in the hippocampus after 2 weeks of daily administration of hypericin. This effect was still obvious after 8 weeks.

Similar results after 2 weeks of daily treatment with a methanolic St John's wort extract were reported by Franklin and Cowen:[72] the extract (20 and 100 mg/kg intraperitoneally) had no effect on serotonin, dopamine and noradrenaline levels in rat whole brain tissues. It can be speculated that a treatment period >2 weeks is required to detect significant changes in brain monoamines, because independent of the administration route (Franklin and Cowen,[72] intraperitoneally vs Butterweck et al.,[71] orally), the same methanolic extract caused no effects. Increasing the treatment period to 8 weeks, Butterweck et al.[71] found that St John's wort extract and hypericin induced a significant reduction of dopamine turnover in the hypothalamus at the end of the treatment period.

The *in vivo* influence of St John's wort on the dopaminergic system has been shown by Winterhoff et al.[73] and Butterweck et al.[74] In *in vitro* experiments, Müller et al.[48] reported that a CO_2 extract and a methanolic St John's wort extract inhibited dopamine reuptake. Moreover, the authors found that hyperforin inhibited dopamine reuptake, whereas the naphthodianthrone hypericin failed to inhibit dopamine reuptake.

A dopaminergic mode of action has also been suggested by Franklin et al.[66,75] and Franklin and Cowen.[72] The authors found that St John's wort extract increased plasma growth hormone levels and decreased plasma prolactin levels in human volunteers after acute administration. Dopaminergic path-

ways facilitate growth hormone release and suppress prolactin secretion.[76]

In conclusion, based on the neurochemical studies, changes in adrenergic, dopaminergic and/or serotonergic neurotransmission may play an important role in mediating the antidepressant effects of long-term administration of St John's wort. However, further pharmacological *in vivo* studies of the effects of long-term administration of St John's wort are necessary to support this hypothesis.

3.5 Effects on Monoamine Receptors

3.5.1 Adrenergic Receptors

A consequence of antidepressant treatment and elevated levels of serotonin or noradrenaline is the occupation of monoamine receptors. One possibility is that persistent activation of these receptors leads to slowly developing receptor adaptations that explain the delayed onset of therapeutic action of antidepressant treatment.[77-79] Early studies demonstrated that long- but not short-term administration of several types of antidepressants decreases β-adrenergic receptor ligand binding in relevant areas such as the cerebral cortex and hippocampus.[80,81]

The question of whether β-adrenoceptor blockade contributes to the effect of St John's wort was first addressed by the study of Müller et al.[37] The authors showed that subacute treatment (14 days) with a methanolic extract of St John's wort (240 mg/kg orally) significantly downregulated the number (B_{max}) of rat cortical β-adrenoceptors without a change in receptor affinity (K_D). The same result – a downregulation of β-adrenoceptors (of 15%) – was achieved using a lipophilic extract obtained with supercritical CO_2 (hyperforin content 38.8%).[48] The authors concluded that hyperforin must be considered a major active antidepressant component of St John's wort. However, the effects of other constituents of St John's wort on β-adrenoceptor downregulation have not been investigated systematically.

3.5.2 Serotonin Receptors

Serotonin has long been implicated in the biological basis of depression as well as in the mechanism of action of antidepressant drugs.[82] The 5-HT_{1A} and 5-HT_2 receptors are candidate targets for antidepressants within the serotonergic system.

Teufel-Mayer and Gleitz[83] showed that long-term administration (26 weeks) of a St John's wort extract (extract not further characterised) upregulated the number of postsynaptic 5-HT_{1A} and 5-HT_{2A} receptors in rat brain with no changes in receptor affinity. The dose of St John's wort extract was 2700 mg/kg. The relevance of these pharmacological effects is therefore questionable, especially because doses above 1500 mg/kg have been proven to be toxic in rats.[84]

An upregulation of 5-HT_2 receptors in the rat frontal cortex after 14 days of daily treatment with 240 mg/kg of a methanolic St John's wort extract was also reported by Müller et al.,[37] whereas a CO_2 extract slightly decreased 5-HT_2 receptor density.[48] Most conventional antidepressants are known to downregulate the expression of 5-HT_2 receptors in rat brain,[85,86] whereas electroconvulsive shock treatment (ECT) causes a significant elevation of 5-HT_2 receptor density.[87] With respect to the upregulation of 5-HT_2 receptors seen by Müller et al.[37] after treatment with St John's wort extract, the action of ECT on 5-HT_2 receptors resembles that of the plant extract. This conformity may indicate that St John's wort and ECT could act by a similar mode of action, although ECT is known to be effective against severe depression whereas St John's wort extracts are indicated for mild to moderate depressive disorders.

In a recent study, Butterweck et al.[27] found that long-term treatment (8 weeks) with imipramine (15 mg/kg orally), St John's wort extract (500 mg/kg orally) and hypericin (0.2 mg/kg orally) decreased 5-HT_{1A} receptor messenger RNA (mRNA) expression in the CA1 of the hippocampus. In the same study, the authors found that long- but not short-term immobilisation stress downregulated 5-HT_{1A} gene expression in the hippocampus. The significant decrease in 5-HT_{1A} gene expression is consistent across various stress experiments.[88,89] Imipramine and St John's wort extract were unable to prevent

the stress-induced decrease in 5-HT_{1A} receptor density.

Data suggest that the common action of antidepressants could be related to an effect on the regulation of the expression of serotonergic receptor subtypes,[90,91] but there is considerable controversy in the literature, particularly using receptor binding studies and behavioural experiments, with regard to the effects of TCAs and SSRIs on 5-HT_{1A} and 5-HT_{2A} receptors, with upregulation, downregulation and no effects being reported.[92-95] None of these mechanisms is completely satisfactory as a common antidepressant mechanism of action. The fact that long-term use of St John's wort produced changes in 5-HT_1 and 5-HT_2 receptors is an interesting pharmacological finding but does not explain the mode of its antidepressant action.

In conclusion, the results presented in sections 3.5.1 and 3.5.2 indicate that the actions of antidepressants in general and St John's wort specifically are probably not mediated only by simple up- or downregulation of monoamines and their receptors.

Data on β-adrenoceptor function and serotonin receptor regulation are summarised in table III.

3.6 Receptor Binding Profile

Several hypotheses have been advanced to explain the therapeutic actions of St John's wort extract on a molecular basis. *In vitro* as well as *in vivo* studies have implicated activity at serotonergic, noradrenergic, dopaminergic and opioid receptors in the hypothesised mechanism of antidepressant action. In this section, the *in vitro* data only will be summarised and discussed. The results from studies of receptor binding are summarised in table IV.

Most *in vitro* research has focused on the crude St John's wort extract.[37,39,47,96,98] There is a paucity of *in vitro* data on single substances, and most authors focused on either a few selected substances[22-24,53,56,100] or individual receptor systems.[47,96,97] One recent study examined the majority of active St John's wort compounds in a wide range of receptor assays to provide a broad *in vitro* pharmacological profile.[28]

3.6.1 St John's Wort Extract

In prior studies, a commercially available crude extract from the fresh flowers and buds of St John's wort was tested in assays in a battery of 39 non-cloned receptors and two enzyme systems.[36] The crude extract of St John's wort had significant affinity for adenosine (subtype unspecified), $GABA_A$ and $GABA_B$, serotonin (subtype unspecified), ben-

Table III. Effects of St John's wort extract and various compounds on β-adrenoceptors and serotonin receptors

Reference	Compound	After 2 weeks	After 8 weeks	After 26 weeks
β-Adrenoceptors				
Müller et al.[37]	Methanolic St John's wort extract	↓	ND	ND
	Imipramine	↓	ND	ND
Müller et al.[48]	CO_2 extract	↓	ND	ND
	Imipramine	↓	ND	ND
Serotonin receptors				
Müller et al.[37]	Methanolic St John's wort extract	↑ (5-HT_2)	ND	ND
Müller et al.[48]	Methanolic St John's wort extract	↑ (5-HT_2)	ND	ND
	CO_2 extract	(↓) [5-HT_2]	ND	ND
Teufel-Mayer and Gleitz[83]	Methanolic St John's wort extract	ND	ND	↑ (5-HT_{1A})
	Methanolic St John's wort extract	ND	ND	↑ (5-HT_{2A})
Butterweck et al.[27]	Methanolic St John's wort extract	–	↓ (5-HT_{1A})	ND
	Hypericin	–	↓ (5-HT_{1A})	ND
	Imipramine	–	↓ (5-HT_{1A})	ND

ND = not determined; **–** indicates no effect; ↓ indicates receptor downregulation; **(↓)** indicates slight receptor downregulation; ↑ indicates receptor upregulation.

zodiazepine and inositol triphosphate (IP_3) receptors and MAO-A and -B. In addition, Simmen and colleagues[96,97] showed that an ethanolic extract inhibited ligand binding to the $GABA_A$ receptor. Baureithel et al.[100] also showed that St John's wort extracts inhibited [^{3}H]flumazenil binding to benzodiazepine binding sites on the $GABA_A$ receptor.

3.6.2 Individual Compounds

Isolated compounds of St John's wort were screened in several studies for activity at G-protein-coupled receptors including serotonin, adrenergic, opioid, histamine, metabotropic glutamate and muscarinic acetylcholine receptors, ligand-gated ion channels including $GABA_{A/B}$ receptors, and various neurotransmitter transporters. The data are contradictory; some of the investigated substances caused an unanticipated binding inhibition in several receptor assays, whereas the same substances were inactive in the same receptor systems in other studies.

In a recent study, the phloroglucinol derivative hyperforin showed considerable affinity for dopamine D_1 and D_5 receptors, whereas the biflavonoid amentoflavone showed affinity for D_3 receptors.[28] Nielsen et al.[101] and Baureithel et al.[100] focused their investigations on the biflavone amentoflavone, which bound to the brain benzodiazepine receptors with an affinity comparable to diazepam. This result was recently confirmed by Butterweck and colleagues.[28] Whereas the flavonoids hyperoside, isoquercitrin, quercitrin, quercetin and miquelianin as well as hyperforin exhibited a weak effect on GABA receptors, the hypericins remained inactive. A similar result was reported by Gobbi et al.,[98] who found that [^{3}H]flumazenil binding to benzodiazepine receptors was inhibited by biapigenin but not by hyperforin or hypericin.

In the study by Butterweck et al.,[28] amentoflavone had remarkable affinity for the δ-opioid receptor subtype in the nanomolar range (K_i = 36.5 nmol/L). The effects of St John's wort extracts and isolated compounds on opioid receptors have been studied recently by Simmen et al.[96,97] Binding of [^{3}H]naloxone to μ- and κ-opioid receptors was inhibited in the presence of St John's wort extracts. Most recently, binding of ligands to μ-, κ- and δ-opioid receptors was found to be inhibited by hyperforin and hypericin in the submicro- and micromolar range.[97] This result could not be confirmed by Butterweck et al.,[28] who found that neither hyperforin nor hypericin affected opioid receptor binding. In agreement with Butterweck et al.,[28] Raffa[53] also found that hypericin had no affinity for opioid receptors.

The most potent effect on GABA receptors was seen for amentoflavone, which inhibited binding of ligands to the benzodiazepine receptor.[28] In the same study, the flavonoids as well as hyperforin exhibited only a weak affinity for GABA receptors, and the hypericins remained inactive. Similar results are reported by Gobbi et al.:[98] [^{3}H]muscimol binding was not inhibited by hypericin, hyperforin or biapigenin.

Data on the effects of hypericin in various receptor-screening models are contradictory: Raffa[53] found that hypericin had no affinity for traditional monoamine receptors or adrenergic, GABA, adenosine or benzodiazepine receptors. The naphthodianthrone had modest affinity for muscarinic cholinergic receptors (subtype not measured) and similar affinity for σ-receptors. Gobbi et al.[98] showed that hypericin inhibited ligand binding to NPY_1, NPY_2 and σ-receptors. The authors found that these inhibitory effects were light dependent, because they decreased or disappeared when binding assays were carried out in the dark.

Cott[36] tested hypericin in a battery of 39 *in vitro* receptor assays, and hypericin showed affinity only for the NMDA receptor (K_i approximately 1 μmol/L). This result could not be confirmed by Butterweck et al.[28] In the same study, hypericin showed high affinity for the D_3 receptor (K_i = 34.5 nmol/L) and negligible affinities (K_i >1000 nmol/L) for nearly all other tested receptors and transporters. Interestingly, the affinity of hypericin for the D_3 receptor subtype was much higher than that of the atypical antipsychotic clozapine (K_i = 372.3 nmol/L).

With regard to the contradictory effects of hypericin in various test models, it should be pointed out that the pharmacological evaluation of hypericin in

Table IV. *In vitro* effects of St John's wort extracts and isolated compounds on various receptors[a]

Receptor subtype	St John's wort extract	CO_2 extract	Hypericin	Pseudo-hypericin	Hyper-forin	Amento-flavone	Biapig-enin	Hypero-side	Isoquer-citrin	Quercitrin	Rutin	Quercetin	Miquelianir
Serotonin													
5-HT_{1A}			–[28]	–[28]	–[28]	–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
5-$HT_{1D\alpha}$			–[28]	–[28]	–[28]	+[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
5-$HT_{1D\beta}$	–[96,97]		–[28]	–[28]	–[28]	–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
5-HT_{2C}			–[28]	–[28]	–[28]	–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
5-HT_3			–[28]	–[28]	–[28]	–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
5-HT_{5a}			–[28]	–[28]	–[28]	–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
5-HT_6	–[98]	–[98]	+[96,97]; –[28,98]	+[96,97]; –[28]	+[96,97]; –[28,98]	–[28]	–[98]	–[28,96,97]	–[28]	–[28]	–[28]	–[28]	–[28]
5-HT_7	–[98]	–[98]	–[28,96-98]	+[96,97]; –[28]	+[96,97]; –[28,98]	–[28]	–[98]	–[28,96,97]	–[28]	–[28]	–[28]	–[28]	–[28]
Dopamine													
D_1	–[36]		–[28,53]	–[28]	+[28]	–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
D_2	–[36]		–[28,53]	–[28]	–[28]	–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
D_3			+[28]	+[28]	–[28]	+[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
D_4			+[28]	+[28]	–[28]	–[28]		–[28]	–[28]	–[28]	–[28]	+[28]	–[28]
D_5			–[28]	–[28]	+[28]	–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
Opioid													
μ	–[96,97]		+[96,97]; –[28,53]	+[96,97]; –[28]	+[96,97]; –[28]	–[28,96]	–[97]	–[28,97,99]	–[28,96,97]	–[28,96,97]	–[28,96,97]	–[28,96,97]	–[28]
δ	–[96,97]		+[96,97]; –[28,53]	+[96,97]; –[28]	+[96,97]; –[28]	+[28]	–[97]	–[28,96,97]	–[28]	–[28]	–[28]	–[28]	–[28]
κ	–[96,97]		+[96,97]; –[28,53]	+[96,97]; –[28]	+[96,97]; –[28]	–[28]	–[97]	–[28,96,97]	–[28,96,97]	–[28]	–[28,96,97]	–[28]	–[28]
Adrenergic													
α_{2A}			–[28]	–[28]		–[28]		+[28]	–[28]	–[28]	–[28]	–[28]	–[28]
α_{2B}			–[28]	–[28]		–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
α_{2C}			–[28]	–[28]		–[28]		–[28]	–[28]	–[28]	+[28]	–[28]	+[28]
β_1	–[36]		–[53]; +[28]	–[28]									
β_2			+[28]	–[28]									
α_{1A}	–[36]		–[28]	–[28]		–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]

Continued next page

Table IV. Contd

Receptor subtype	St John's wort extract	CO_2 extract	Hypericin	Pseudo-hypericin	Hyper-forin	Amento-flavone	Biapig-enin	Hypero-side	Isoquer-citrin	Quercitrin	Rutin	Quercetin	Miquelianir
α_{1B}			–[28]	–[28]		–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
Acetylcholine													
M_1			–[28]	–[28]		–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
M_2			–[28]	–[28]		–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	+[28]
M_3			–[28]	–[28]		–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
M_4			–[28]	–[28]		–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
M_5			–[28]	–[28]		–[28]		–[28]	–[28]	–[28]	+[28]	–[28]	+[28]
GABA													
$GABA_A$	+[36,96-98]	–[98]	–[28,36,53,98]		–[28,98]	–[28]	–[98]	–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
$GABA_B$	+[36]		–[36]										
Benzodiazepine	+[36,100]; –[98]	–[98]	–[28,36,53,98,100]	–[28]	–[28,98]	+[28,100,101]	+[98]; –[100]	–[28]	–[28]	–[28,100]	–[28,100]	–[28,100]	–[28]
Glutamate													
NMDA	–[36]		–[28,53]; +[36]	–[28]	–[28]	–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
Phencyclidine			–[28]	–[28]	–[28]	–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
Histamine													
H_1	–[96,97]		–[53]		–[28]	–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
H_2	–[96,97]												
Peptides													
V_1			–[28,53]	–[28]	–[28]	–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
V_2			–[28]	–[28]	–[28]	–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
V_3			–[28]	–[28]	–[28]	–[28]		–[28]	–[28]	–[28]	–[28]	–[28]	–[28]
$NPY_{1,2}$	–[98]	–[98]	+[98]; –[53]		–[98]		–[98]						
NK_1			–[53]										
CRF_1			+[97]	+[97]	–[97]								
Estrogen-α	–[96,97]						+[96,97]			–[97]		–[98]	
σ	–[98]	–[98]	+[53,98]				–[98]						

a This table presents an overview of receptor binding studies but does not consider quantitative differences, because the original data from different authors had a different 'cut-off' for activity.

CRF = corticotrophin release factor; **M** = muscarinic; **NK** = neurokinin; **NPY** = neuropeptide Y; **V** = vasopressin; **+** indicates affinity for the receptor; **–** indicates no affinity for the receptor.

most *in vitro* and *in vivo* studies is hampered by its poor solubility in most aqueous solutions. This important fact will be discussed in more detail in section 4.

Taken together, what relevance do the summarised *in vitro* receptor binding findings have for understanding the mode of action of St John's wort? Clearly, because related compounds interact with various receptors, it can be hypothesised that additive or synergistic effects induced by distinct compounds at multiple targets might be responsible for the antidepressant efficacy of St John's wort. However, the concentration of compounds required for half-maximal receptor occupancy in all mentioned studies appears to be quite high. Circulating concentrations that are within two orders of magnitude of the K_i values may be sufficient for subtle modulation of synaptic transmission.[102,103] A further limitation is that studies of intrinsic activity have not been done. In addition, pharmacokinetic data for relevant substances are needed. *In vivo* studies are necessary to establish the pharmacological relevance of these *in vitro* findings for therapeutic usage of St John's wort.

4. Behavioural Pharmacology in Animals

The potential antidepressant activity of St John's wort was confirmed in a number of animal models of depression.

Human psychiatric disorders cannot be induced in animals, so animal models cannot be claimed to reproduce human psychopathology. Instead, they are intended to induce changes that are sensitive to therapeutic agents in a manner predictive of their effects in humans.[104] In this review, two animal models are of particular interest: (i) the forced swimming test (FST) ['behavioural despair'];[105] and (ii) the learned-helplessness paradigm.[106]

4.1 Forced Swimming Test

The FST was developed by Porsolt et al.[105] The authors described procedures for simulating a state of 'depression' in rats. There is, in fact, a significant correlation for antidepressants between clinical potency and their ability to reduce immobility time in the FST.[107] The specificity of this test for antidepressants has been questioned because a large number of nonantidepressants also reduce immobility. False positive results have been reported for stimulants, anticholinergics, antihistamines, pentobarbital (phenobarbitone) and opiates.[107-111] It has been found that whereas response to antidepressants persists with long-term treatment, it disappeared with long-term administration of antihistamines.[112] The effects of stimulants such as caffeine or amphetamine can be excluded by additional open field tests, in which these agents show marked increases in locomotor activity.[113] Thus, the FST, when validated, appears particularly useful for the screening of antidepressant drugs.[114]

In the FST, extracts of St John's wort have consistently been shown to decrease immobility time dose dependently after single-dose as well as after repeated treatment[20,22,26,29,73,115-117] without changing locomotor activity.[71,73] In all studies, the efficacy of St John's wort extract was comparable to that of standard antidepressants. The results in the FST after repeated treatment and after additional open field tests confirm a specific antidepressant effect of the plant extract.

During a bioassay-guided fractionation of a methanolic St John's wort extract, two main fractions were obtained that significantly decreased immobility time in the FST.[20] These fractions were characterised by quantitative HPLC as being rich in hypericin, pseudohypericin and procyanidins (fraction IIIc) and flavonoids (fraction II).[20] Further investigations focused on isolated compounds from these fractions. Interestingly, pure hypericin and pseudohypericin did not reduce immobility time after single-dose pretreatment at doses comparable to the entire extract; only the exceptionally high dose of hypericin 0.23 mg/kg (orally) was significantly active, whereas pseudohypericin indicated some nonsignificant activity at about 0.5 mg/kg (orally).[26] When the fraction of procyanidins, which was itself not active in the FST, was recombined with the naphthodianthrones, hypericin was significantly active at 0.028 mg/kg (orally) and

pseudohypericin was active at 0.166 mg/kg (orally). The procyanidin fraction as well as the pure procyanidins B2 and C1 increased the water solubility of both hypericins, which may lead to a better bioavailability.[26] However, after repeated treatment (14 days), hypericin was active in the FST without supplementation of procyanidin B2,[74] indicating that when hypericin is administered repeatedly, the endogenous level will reach pharmacodynamic concentrations without a solubiliser. These interesting pharmacokinetic properties could also explain why hypericin was inactive in most *in vitro* experiments: the biological evaluation of hypericin in various tests is hampered by its poor water solubility, which can be increased with the presence of various procyanidins (see also section 3.6).

The bioassay-guided fractionation yielded a fraction that was mainly characterised by its high number of flavonoids. The fraction significantly reduced immobility time in the FST, and the effect was comparable to that of imipramine.[20] The flavonoid fraction was further purified and some flavonol glycosides such as hyperoside, quercitrin, isoquercitrin and miquelianin, as well as the flavone glycoside astilbin, were isolated[21] and tested for activity in the FST at doses comparable to their amounts present in the crude drug material.[29] Except for quercitrin and astilbin, all flavonoids (hyperoside, isoquercitrin and miquelianin) were significantly active in the FST after single-dose as well as after repeated treatment (oral administration). In addition, no increase of locomotor activity was observed, indicating that their effects in the FST were specific. The aglycone quercetin did not show activity in the FST when tested at 0.4 mg/kg (orally), a dose analogous to hyperoside. The data obtained by Butterweck et al.[29] indicate that certain flavonol-3-O-glycosides are active constituents of the St John's wort extract. The activity seems to be bound to the sugar moiety of the aglycone quercetin, in that its glucoside, galactoside and glucuronide are active compounds. It may be that these particular glycosides are absorbed from the intestine, whereas others are not. Additional studies are necessary to verify this hypothesis.

Chatterjee and coworkers[22] have recently pointed to hyperforin as the major non-nitrogenous compound of St John's wort. Oral administration of an ethanolic extract (hyperforin content 4.5%; dose range 50–300 mg/kg) and a supercritical CO_2 extract (hyperforin content 38.8%; dose range 5–30 mg/kg) dose dependently reduced immobility time of rats in the FST. Based on these results, the authors conclude that "hyperforin is the major active compound of the extract".[22] Unfortunately, pure hyperforin was not tested in this study; theoretical dose-effect curves of hyperforin were calculated on its concentration in the tested extracts. These mathematical calculated results nicely show that the (theoretical) activity of hyperforin in the FST correlates well with its concentration in the different extracts. Since the pure compound was not tested in the FST in that study, it cannot be excluded that the effects of the CO_2 extract are due to effects of so-far untested compounds (e.g. adhyperforin).

Taken together, the data indicate that the FST has been proven to be a reliable animal model for the evaluation of substances with antidepressant activity. Extracts of St John's wort (methanolic, ethanolic and CO_2 extracts) as well as pure compounds such as hypericin and several flavonoids have been proven to be active in this model, with effects comparable to standard antidepressants.

4.2 Learned-Helplessness Paradigm

The learned-helplessness paradigm was originally described by Overmier and Seligman[106] in dogs, and it was then extended to a large number of other species including rats and humans.[118] According to this paradigm, exposure to uncontrollable stress produces performance deficits in subsequent learning tasks that are not seen in subjects exposed to the identical stressor but able to control it.[118,119] Learned helplessness could be reversed by subchronic treatment (4–7 days) with a variety of antidepressants including TCAs, MAO inhibitors and atypical antidepressants and with electroconvulsive shocks.[120-123]

The learned-helplessness paradigm responds to a wide range of clinically effective treatments, and

there are no false negatives. However, the effects of either long-term anticholinergic treatment or antihistamines on learned helplessness have not been assessed.[124] Also, the specificity of learned helplessness as a model of depression has been questioned.[104] Therefore, there are serious doubts regarding the validity of learned helplessness as a specific model of depression; its specificity is unclear, and the model appears to predict patterns of symptoms that are not found to occur in depression.[125]

Chatterjee and colleagues[99] were the first to investigate two different St John's wort extracts in the learned-helplessness paradigm. In this study, the escape failures in the group treated with ethanolic extract (hyperforin content 4.5%) significantly and dose dependently decreased after 150 and 300 mg/kg/day (orally). Comparable effects were observed for the CO_2 extract (hyperforin content 38.8%) at dosages of 15 and 30 mg/kg/day (orally).

A similar study was performed by Gambrana et al.[126] The authors studied the effects of a hydroalcoholic St John's wort extract (hypericin content 0.3%, hyperforin content unknown) on the escape-deficit paradigm. The escape-deficit paradigm is a simplified version of the classical learned-helplessness syndrome.[106,118,123] St John's wort extract, given as a single dose at relatively high doses of 1–1.5 g/kg (orally), significantly protected rats from the consequences of exposure to acute unavoidable stress. Chronic stress induced a long-lasting escape deficit that was completely reversed by repeated pretreatment with imipramine, fluoxetine or St John's wort extract. The authors further studied the efficacy of a CO_2 extract (extract not further characterised) in the acute escape deficit model after single and repeated treatment.[127] A dose-response curve for the CO_2 extract, administered orally acutely before stress exposure, showed that this preparation was five times more potent than the total extract (extract not further characterised). Pure hyperforin was also tested in the acute escape-deficit paradigm in a dose range of 12.5–75 mg/kg (intraperitoneal injection). Hyperforin 20, 50 and 75 mg/kg significantly counteracted the effects of acute stress. The authors concluded that hyperforin when administered intraperitoneally is several times more potent than the total hydroalcoholic extract when given orally.

Taken together, the results in the learned-helplessness paradigm show that different St John's wort extracts (hydroalcoholic, CO_2) as well as pure hyperforin can reduce behavioural deficits induced by uncontrollable stress. How active other constituents of St John's wort in this model are needs to be further elucidated.

The data of the FST and the learned-helplessness paradigm are summarised in table V.

5. Other Possible Modes of Action

A relationship between endocrine disorders and depression has been noted for many years, and data indicate functional disturbances of endocrine systems in depression. The hypothalamic-pituitary-adrenal (HPA) axis, the hypothalamic-pituitary-thyroid (HPT) axis, the neuropituitary axis and the somatotropic axis are particularly affected.[128-131] A common biological alteration in patients with major depression is activation of the HPA axis, which

Table V. Effects of St John's wort extract and various compounds in the forced swimming test and learned-helplessness paradigm

Substance	Forced swimming test	Learned helplessness
Hydroalcoholic St John's wort extract	↓[20,22,26,29,73,74,115-117]	↓[22,126,127]
CO_2 extract	↓[22]	↓[22,126,127]
Flavonoid fraction (II)	↓[20,29]	ND
Hypericin + procyanidin fraction (IIIc)	↓[20,26]	ND
Pure hypericin	–[26]	ND
Pure pseudohypericin	–[26]	ND
Procyanidin B2	–[26,74]	ND
Pure hypericin + procyanidin B2	↓[26,74]	ND
Hyperoside	↓[29]	ND
Quercitrin	–[29]	ND
Isoquercitrin	↓[29]	ND
Miquelianin	↓[29]	ND
Astilbin	–[29]	ND
Hyperforin	ND	↓[127]
Imipramine	↓[20,22,26,29,73,74,115-117,126,127]	↓[22,126,127]

ND = not determined; ↓ indicates decrease of immobility time/escape deficit; – indicates no effect.

manifests itself as hypersecretion of adrenocorticotrophic hormone (ACTH) and cortisol and an abnormal cortisol response to dexamethasone and corticotrophin-releasing hormone (CRH).[128,132,133] Correspondingly, the hyperactivity of the HPA axis in depressed patients can be corrected by effective therapy with antidepressant drugs.[134,135] Similarly, chronic stress in animals results in elevated ACTH and glucocorticoid levels in plasma and elevated production of CRH in the parvocellular portion of the hypothalamic paraventricular nucleus (PVN).[136-138]

Administration of antidepressants influences the expression of genes coding for HPA-axis constituents. In an animal study designed to examine the association between long-term antidepressant administration and the possibly delayed alteration in HPA axis activity, CRH mRNA in the PVN of rats was shown to be decreased following long-term (8 weeks) but not short-term (2 weeks) treatment with imipramine, the prototypic TCA.[69] The same results were found with several other antidepressant drugs selected for their distinctly different primary pharmacological actions.[70]

Based on the results of Brady et al.,[69,70] Butterweck et al.[27] performed a study to examine in rats the effects of short-term (2 weeks) and long-term (8 weeks) administration of imipramine, St John's wort extract and hypericin on the expression of genes that may be involved in the regulation of the HPA axis. Imipramine (15 mg/kg orally), St John's wort (500 mg/kg orally) and hypericin (0.2 mg/kg orally) significantly decreased levels of CRH mRNA by 16–22% in the PVN when given daily for 8 weeks but not for 2 weeks. Comparable to imipramine, both the extract and hypericin reduced plasma ACTH and corticosterone levels after 2 weeks of daily treatment.

Franklin et al.[66] found increased plasma corticosterone levels in rats after single doses of St John's wort extract (200 mg/kg intraperitoneally), hypericin (0.2 mg/kg intraperitoneally) and hyperforin (9.3 mg/kg intraperitoneally). Median maximal plasma levels were observed 2 hours after injection. However, a vehicle-treated control group was not included in this study, so it is not clear if the observed increase in plasma corticosterone level was due to the treatment or induced by stress during the treatment procedure (intraperitoneal injection). For example, similar increased corticosterone levels were observed after acute immobilisation stress.[27]

The study by Butterweck et al.[27] showed that the three compounds – imipramine, St John's wort extract and hypericin – all produced similar delayed changes in CRH gene transcription, suggesting a shared action on the centres that control HPA axis activity. The data from Butterweck et al.[27] also suggest that the delayed changes may be important for the therapeutic efficacy of St John's wort extract and hypericin. This hypothesis is based on changes in mRNA expression in unstressed, eucortisolaemic rats.

The authors performed a second experiment to assess the effects of long-term administration of imipramine and St John's wort extract on stress-induced changes in gene transcription in stress-responsive circuits.[27] Depression-associated behavioural symptoms can be observed in stress models, and long-term but not acute administration of antidepressants usually reverses these effects.[125,139,140] However, Butterweck et al.[27] found that repeated immobilisation stress (2 hours daily for 7 days) increased CRH mRNA levels in the PVN, pro-opiomelanocortin (POMC) in the anterior pituitary, glutamic acid decarboxylase (GAD 65/67) in the bed nucleus of the stria terminalis (BST), cyclic adenosine monophosphate response element binding protein (CREB) in the hippocampus and tyrosine hydroxylase in the locus coeruleus. It decreased levels of mRNA for 5-HT_{1A} receptors and brain-derived neurotrophic factor (BDNF) in the hippocampus. Long-term pretreatment with either imipramine or St John's wort reduced to control levels the stress-induced increases in gene transcription of GAD in the BST, CREB in the hippocampus and POMC in the pituitary. The stress-induced increases in CRH mRNA in the PVN and tyrosine hydroxylase in the locus coeruleus were reduced by imipramine but not by St John's wort. The stress-induced

decreases in BDNF and 5-HT_{1A} mRNA were not prevented by either drug.

The data of Butterweck et al.[27] show that (i) St John's wort extract and hypericin have delayed effects on HPA axis control centres similar to those of imipramine; and (ii) selective stress-induced changes in gene transcription in particular brain areas can be prevented by long-term treatment with either the prototypic TCA imipramine or St John's wort. However, imipramine appeared to be more effective in blocking stress effects on the HPA axis than the plant extract.

Further evidence that St John's wort extract interacts with certain neuroendocrine pathways was reported by Thiele and colleagues.[141] The authors investigated *in vitro* the effect of St John's wort extract on lipopolysaccaride-stimulated whole blood culture systems from five healthy volunteers and four depressed patients. The release of interleukin (IL)-6, IL-1β and tumour necrosis factor-α (TNFα) was measured. Whereas St John's wort extract massively suppressed IL-6 release from stimulated blood cells, no effect was observed on IL-1β and TNFα. Similar results were obtained by Fiebich et al.[142] The authors showed that different extracts from St John's wort dose dependently inhibited substance P-induced IL-6 synthesis in a human astrocytoma cell line. Substance P is a neuropeptide that is involved in the aetiology of neurogenic inflammation and, as discovered more recently, of depression and anxiety.[143]

Both clinical and experimental studies indicate that stress and depression are associated with increased circulating levels of cytokines such as IL-1β and IL-6 and hyperactivity of the HPA axis. It is well established that HPA-axis activation is elicited by exogenous cytokine administration to rodents. Thus, it was suggested that hypercortisolaemia observed in depressed patients might have resulted from a hypersecretion of CRH induced by pro-inflammatory cytokines such as IL-1β or IL-6. It is therefore not unreasonable to speculate that the St John's wort-induced IL-6 inhibition may be responsible for the decreased CRH expression. This aspect could be of relevance for the mechanism of antidepressant action of St John's wort. Further evidence is necessary to support this hypothesis.

6. Concluding Remarks

Herbal medicines are complex mixtures of more than one active ingredient. Therefore, pharmacological work is complicated by the fact that active compounds are often unknown. Further, synergistic or antagonistic effects of the different compounds cannot be excluded. Nevertheless, the example of St John's wort offers convincing proof for the concept that modern methods of pharmacological, phytochemical, biochemical and molecular biological research are effective in advancing the development of traditional herbal remedies with CNS activity. As a consequence of these efforts, today several compounds from different structural groups and with different mechanisms of action seem to be responsible for the observed antidepressant efficacy of St John's wort. Based on recent research, it seems therefore likely that flavonoids, hyperforins and hypericins contribute to the antidepressant efficacy of St John's wort. Their presence in a sufficient amount in St John's wort extracts should be guaranteed to provide herbal products with a high pharmaceutical quality.

Research on the active constituents of St John's wort is far from finished. Furthermore, the mechanism of antidepressant action of the plant extract is still not fully understood. Our understanding of the mode of action of St John's wort is complicated by the fact that the molecular basis of depression itself is still unclear. More than 50 years after the discovery of the first antidepressants, new findings and new hypotheses are still being reported in an attempt to provide more insight into the mode of action of these substances. Considering the relatively short time that St John's wort has been under systematic investigation, there has been much progress in our understanding of its CNS actions, but there is still a lot to be done.

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