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# Effects of oral administration of extracts of *Hypericum perforatum* (St John's wort) on brain serotonin transporter, serotonin uptake and behaviour in mice

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## Abstract

The pharmacological effects of extracts of *Hypericum perforatum* (St John's wort) were characterized in-vitro and ex-vivo, in relation to its behavioural effects. In in-vitro experiments, St John's wort inhibited brain synaptosomal [$^3$H]serotonin uptake in mice with little effect on specific [$^3$H]paroxetine binding. For selective serotonin-reuptake inhibitors (SSRIs), the IC50 value for [$^3$H]serotonin uptake (molar concentration of unlabelled drug necessary to displace 50% of specific uptake) correlated well with the inhibition constant $K_i$ value for [$^3$H]paroxetine binding in mouse brain. Oral administration of St John's wort (900 mg kg$^{-1}$), paroxetine (1 mg kg$^{-1}$) and sertraline (10 mg kg$^{-1}$) brought about significant increases in the $K_m$ value for [$^3$H]serotonin uptake into brain synaptosomes 4 h later, and only SSRIs suppressed specific [$^3$H]paroxetine binding in mouse brain. St John's wort and SSRIs significantly inhibited marble-burying behaviour in mice and the time-course of attenuation of this behaviour by St John's wort was similar to that of [$^3$H]serotonin uptake inhibition. In the forced swimming test, St John's wort, but not SSRIs, suppressed the immobility time of mice after oral administration. These results provide the first in-vivo evidence to suggest that the mode of antidepressant action of St John's wort differs from that of SSRIs. Thus, this study may have a significant impact on phytotherapy with St John's wort.

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## Introduction

Extracts of *Hypericum perforatum* (St John's wort) are frequently prescribed in Germany and other European countries in the treatment of mild to moderate depression, anxiety and sleep disorders. Several recent reviews of controlled clinical studies with St John's wort have come to the conclusion that it represents an effective antidepressive principle, superior to placebo (Linde et al 1996; Volz 1997; Wheatley 1997; Wong et al 1998) and having a similar effect to some standard antidepressant drugs (Philipp et al 1999; Brenner et al 2000; Schrader 2000; Woelk 2000). Furthermore, this extract has proved to be free of cardiac and anticholinergic side effects, which are typical for the tricyclic antidepressants (Ernst et al 1998). In accordance with the clinical studies, many recent pharmacological studies with St John's wort also support its antidepressive activity, and attention has increasingly focused on hyperforin (a phloroglucinol derivative) as an active ingredient. St John's wort and hyperforin (80–200 nM) inhibit synaptosomal monoamine uptake in-vitro (Chatterjee et al 1998), and these hyperforin concentrations are close to its plasma $C_{max}$ value in human subjects given daily St John's wort (300 mg of the extract containing 5% hyperforin) (Biber et al 1998). In in-vivo experiments, acute systemic treatment with St John's wort exerts significant antidepressant activity in some behavioural tasks (forced swimming test, learned helplessness and tail suspension test) (Chatterjee et al 1998; Butterweck et al 2003) and St John's wort (500 mg kg$^{-1}$, p.o.) or hyperforin alone (10 mg kg$^{-1}$, i.p.) significantly enhances levels of brain serotonin, noradrenaline (norepinephrine) and dopamine in rats (Calapai et al 1999; Kaehler et al 1999).

Although a wide variety of bioactive compounds, such as phenylpropanes, flavonol derivatives, biflavones, proanthocyanidines, xanthones, phloroglucinols, some amino

acids, naphthodianthrones and essential oil constituents, have been identified in the extracts (Nahrstedt & Butterweck 1997), it is still not clear which components could account, wholly or partly, for the antidepressant activity of St John's wort. Because elimination of hyperforin from St John's wort does not result in a loss of behavioural pharmacological activity (Butterweck et al 2003) and the brain-to-plasma ratio of hyperforin is only 4%, the corresponding brain concentration of hyperforin is probably far from the levels required to affect the neurotransmitter mechanisms (Cervo et al 2002). Thus, the exact mechanism by which St John's wort exerts its antidepressant effect still remains to be resolved. Most previous studies on St John's wort and its ingredients have involved in-vitro experiments and behavioural effects but little in-vivo study has been undertaken to establish the pharmacological relevance of in-vitro findings. Therefore, it would be of general importance to elucidate the in-vivo mode of action of St John's wort under the influence of pharmacokinetic and pharmacodynamic factors. In this study, the binding characteristics of St John's wort to serotonin transporters and its effects on serotonin uptake in mouse brain were examined in-vitro and ex-vivo, in relation to its behavioural effects. Mice received St John's wort (100–900 mg kg$^{-1}$), paroxetine (1 mg kg$^{-1}$) and sertraline (10 mg kg$^{-1}$) orally at doses chosen to significantly increase the extracellular serotonin in rat brain (Calapai et al 1999; Malagié et al 2000; Bymaster et al 2002).

## Materials and Methods

### Chemicals

[$^3$H]Paroxetine (806.6 GBq mmol$^{-1}$) and [$^3$H]serotonin (1.0 TBq mmol$^{-1}$) were purchased from Dupont-NEN Co. Ltd (Boston, MA). The *Hypericum perforatum* dry extract (St John's wort) was kindly supplied by Indena (Milan, Italy). The content of hypericin (0.3%) and hyperforin (3.2%) was quantified by Indena. Fluvoxamine maleate was purchased from Tocris (UK). Fluoxetine hydrochloride, paroxetine hydrochloride and sertraline hydrochloride were kindly donated by Eli Lilly pharmaceuticals (Greenfield, IN), GlaxoSmithKline pharmaceuticals (West Sussex, UK) and Pfizer Inc. (Groton, CT), respectively. All other drugs and materials were obtained from commercial sources.

### Drug administration

Male ICR strain mice, 6–8 weeks old (Japan SLC Inc., Shizuoka, Japan), were housed five per cage in the laboratory with free access to food and water, and were maintained on a 12-h dark–light cycle in a room with controlled temperature (24 ± 1°C) and humidity (55 ± 5%). Mice were fasted for 16 h before the administration of drugs. St John's wort (100, 300 and 900 mg kg$^{-1}$), paroxetine (1 mg kg$^{-1}$) or sertraline (10 mg kg$^{-1}$) was administered orally, and control mice received vehicle. St John's wort was suspended in distilled water and sonicated for 10 min before oral administration; SSRIs were dissolved in distilled water. At 1–12 h after the administration, mice were exsanguinated by taking the blood from the descending aorta under light anaesthesia with diethyl ether and the brain was perfused with 0.9% NaCl from the aorta. Then, the whole brain was removed and used for [$^3$H]paroxetine binding or [$^3$H]serotonin uptake experiments. All the procedures used in this study were conducted according to guidelines approved by the Experimental Animal Ethical Committee of the University of Shizuoka.

### [$^3$H]Paroxetine binding assay

The whole brain tissue was homogenized in 19 volumes of 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl with a Polytron homogenizer and the homogenate was centrifuged at 40 000 *g* for 15 min. The pellet was resuspended in 24 volumes of the buffer. All steps for the tissue preparation were performed at 4°C. The binding assay for serotonin transporters in brain homogenates from mice was performed by using [$^3$H]paroxetine, as previously described (Habert et al 1985). Briefly, the brain homogenates (approximately 400 $\mu$g of protein) were incubated with different concentrations of [$^3$H]paroxetine (0.1, 0.3, 0.5, 1.0 and 2.0 nM) for 2 h at 20°C in the buffer. The reaction was terminated by rapid filtration (Cell Harvester; Brandel Co., Gaithersburg, MD) through Whatman GF/B glass fibre filters, and filters were rinsed three times with 2 mL of ice-cold buffer. Tissue-bound radioactivity was extracted from filters overnight in scintillation fluid (2 L of toluene, 1 L of Triton X-100, 15 g of 2,5-diphenyloxazole, 0.3 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene) and it was determined in a liquid scintillation counter. Specific binding of [$^3$H]paroxetine was determined experimentally from the difference between counts in the absence and presence of 10 $\mu$M fluoxetine. All assays were conducted in duplicate. Every binding experiment was performed using fresh tissues. Protein concentration was measured according to the method of Lowry et al (1951) using bovine serum albumin as standard.

### Synaptosomal uptake of [$^3$H]serotonin

Synaptosomal preparations from the whole brain of mice were used for serotonin uptake as previously described (Chatterjee et al 1998; Singer et al 1999). The tissue was homogenized in 0.32 M sucrose solution with a Teflon–glass homogenizer and diluted with 10 mL of homogenizing medium. The nuclear fraction was eliminated by centrifugation at 750 *g* for 10 min and the supernatant was centrifuged at 17 400 *g* for 20 min to obtain the crude synaptosomal pellet. The pellet was suspended in 19 volumes of HEPES buffer (composition in mM (except where stated): 10 HEPES, 150 NaCl, 6.2 KCl, 1.2 $Na_2HPO_4$, 1.2 $MgSO_4$, 10 glucose, 10 $\mu$M pargylin, 0.1% ascorbic acid, pH 7.4). All steps for the tissue preparation were performed at 4°C. The sample of suspension was incubated at 37°C for 15 min and cooled on ice. [$^3$H]Serotonin (10, 20, 30, 50 and 100 nM) was added and the uptake started by incubation at 37°C for 4 min, during

which time the uptake is linearly dependent on time. The reaction was terminated by rapid filtration and radioactivity was determined in a liquid scintillation counter as described above. Nonspecific uptake of [$^3$H]serotonin was determined in the presence of 500 $\mu$M serotonin and specific uptake was expressed as fmol min$^{-1}$ ($\mu$g protein)$^{-1}$. All assays were conducted in duplicate.

### Behavioural test

Mice received St John's wort (100, 300 and 900 mg kg$^{-1}$), paroxetine (1 mg kg$^{-1}$) or sertraline (10 mg kg$^{-1}$) orally and control mice received vehicle. At 1–12 h after the administration, mice were subjected to the forced swimming test or marble-burying test. The forced swimming test was essentially similar to that described previously (Porsolt et al 1978). A glass cylinder (height 25 cm, diameter 10 cm) was utilized and this was filled with water maintained at 23°C (depth 10 cm). Mice were dropped individually into the cylinders, and left there for 6 min. A mouse was judged to be immobile when it floated in an upright position and made only small movements to keep its head above water. The total immobility time(s) of each mouse was expressed as the sum of the immobility periods observed during the 6-min forced swimming test.

For the marble-burying test, an open cubic transparent plastic box (22.5 × 33.8 × 14.0 cm) was used, and 20 clean glass marbles (15 mm diameter) were evenly spaced (5 cm apart) on sawdust (5 cm deep) as previously described (Njung'e & Handley 1991; Ichimaru et al 1995). Mice were placed into the cubic box individually for 30 min and the number of marbles left uncovered was counted. The results of the marble-burying test were expressed as the number of marbles covered with sawdust by mice during 30-min testing period.

The locomotor activity of mice was measured for 30 min by an activity sensor (NS-AS01; Neuroscience Inc., Tokyo, Japan) in the same apparatus utilized for the marble-burying test (without marbles and sawdust) as a separate experiment. The results were represented as activity counts (the number of movement of mice) during the 30-min testing period.

### Data analysis

Analysis of binding data was performed as described previously (Yamada et al 1980). The apparent dissociation constant ($K_d$) and maximal number of binding sites ($B_{max}$) for [$^3$H]paroxetine were estimated by Rosenthal analysis of the saturation data (Rosenthal 1967). Kinetic parameters ($K_m$ and $V_{max}$) were calculated using Lineweaver–Burk plots. The ability of St John's wort and SSRIs to inhibit specific [$^3$H]paroxetine (0.3 nM) binding and [$^3$H]serotonin (10 nM) uptake in-vitro was estimated by IC50 value, which is the molar concentration of unlabelled drug necessary for displacing 50% of specific binding or uptake (estimated by log probit analysis). The inhibition constant, $K_i$, was calculated from the equation, $K_i = IC50/(1 + L/K_d)$, where L is the concentration of the radioligand.

Statistical analysis was performed by the non-parametric Kruskal–Wallis test followed by Dunn's post test for multiple comparisons utilizing the Prism 4.0 program (GraphPad Inc., San Diego, CA). The number of determinations (n) was noted in each figure and table, and the level of $P < 0.05$ was considered significant.

## Results

### In-vitro inhibitory effect of St John's wort and SSRIs on specific [$^3$H]serotonin uptake and [$^3$H]paroxetine binding in mouse brain

The [$^3$H]serotonin (10–100 nM) uptake and [$^3$H]paroxetine (0.1–2.0 nM) binding in mouse brain were saturable with $K_m$ and $K_d$ values of 21.5 and 0.13 nM, respectively, in accordance with previous observations (Habert et al 1985; Singer et al 1999). The total uptake of [$^3$H]serotonin into brain synaptosomes was reduced to a nonspecific level by incubation at 4°C or by the presence of 10 $\mu$M fluoxetine instead of 500 $\mu$M serotonin. St John's wort (hyperforin equivalent: 30–300 nM) and fluvoxamine (1–100 nM), fluoxetine (1–100 nM), paroxetine (0.1–10 nM) and sertraline (1–100 nM) inhibited synaptosomal uptake of [$^3$H]serotonin in a concentration-dependent manner and the inhibitory effect was in the order: paroxetine > sertraline > fluvoxamine > fluoxetine >> St John's wort. For St John's wort, both the IC50 for [$^3$H]serotonin uptake and the $K_i$ for [$^3$H]paroxetine binding were expressed as the corresponding concentration of hyperforin calculated from the amount (3.2%) of this constituent (Table 1). IC50 values of SSRIs for [$^3$H]serotonin uptake were similar to their $K_i$ values for [$^3$H]paroxetine binding. On the other hand, St John's wort exerted little inhibitory effect on specific [$^3$H]paroxetine binding in-vitro.

### Effect of oral administration of St John's wort and SSRIs on specific [$^3$H]serotonin uptake and [$^3$H]paroxetine binding

The effect of oral administration of St John's wort and SSRIs on [$^3$H]serotonin uptake into mouse brain synaptosomes

**Table 1** In-vitro inhibition by St John's wort and SSRIs of specific [$^3$H]serotonin uptake and [$^3$H]paroxetine binding in mouse brain

| Drug | IC50 (nM) for [$^3$H]serotonin uptake | $K_i$ (nM) for [$^3$H]paroxetine binding |
|---|---|---|
| St John's wort (by hyperforin equiv.) | 179 ± 8 (4) | > 1000 (4) |
| Fluvoxamine | 6.29 ± 1.40 (4) | 5.52 ± 0.82 (3) |
| Fluoxetine | 12.8 ± 1.2 (4) | 10.8 ± 1.9 (3) |
| Paroxetine | 0.17 ± 0.05 (4) | 0.54 ± 0.04 (3) |
| Sertraline | 3.11 ± 0.18 (4) | 3.39 ± 1.25 (3) |

Values for St John's wort are expressed as hyperforin content of the 3.2% extract. Values are mean ± s.e. of 3 or 4 mice (no. of replicates given in parentheses).

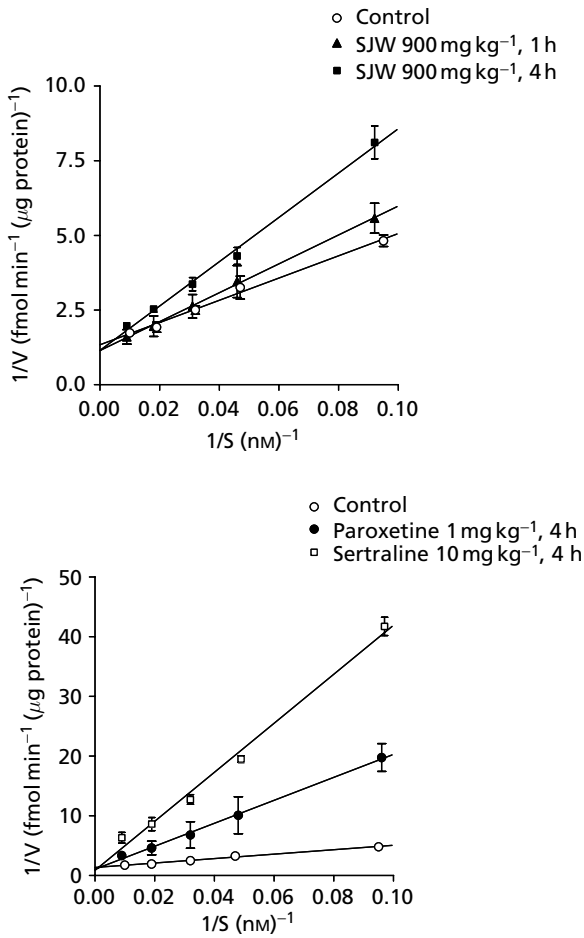


**Figure 1** Lineweaver–Burk analysis for synaptosomal [$^3$H]serotonin uptake in mouse brain after oral administration of St John's wort (SJW), paroxetine and sertraline. Mice received St John's wort (900 mg kg$^{-1}$), paroxetine (1 mg kg$^{-1}$) or sertraline (10 mg kg$^{-1}$), and were exsanguinated by taking blood from the descending aorta at 1 or 4 h after the administration. Specific [$^3$H]serotonin (10–100 nM) uptake into brain synaptosomes was measured. Each point represents mean ± s.d. of 4 mice.

**Table 2** Effect of oral administration of St John's wort and SSRIs on the $K_m$ and $V_{max}$ values for specific [$^3$H]serotonin uptake into mouse brain synaptosomes

| Drug | n | Time (h) | $K_m$ (nM) | $V_{max}$ (fmol min$^{-1}$ (μg protein)$^{-1}$) |
|---|---|---|---|---|
| Control | 4 | | 27.9 ± 1.2 | 0.75 ± 0.02 |
| St John's wort 300 mg kg$^{-1}$ | 4 | 4 | 30.1 ± 0.1 | 0.82 ± 0.02 |
| St John's wort 900 mg kg$^{-1}$ | 4 | 1 | 35.0 ± 1.0 | 0.82 ± 0.05 |
| St John's wort 900 mg kg$^{-1}$ | 4 | 4 | 49.6 ± 4.2* | 0.75 ± 0.02 |
| St John's wort 900 mg kg$^{-1}$ | 3 | 12 | 27.4 ± 4.0 | 0.75 ± 0.06 |
| Paroxetine 1 mg kg$^{-1}$ | 4 | 4 | 178 ± 37* | 0.92 ± 0.06 |
| Sertraline 10 mg kg$^{-1}$ | 4 | 4 | 208 ± 34** | 0.57 ± 0.01* |

Values are means ± s.e. of 3 or 4 mice. *$P < 0.05$, **$P < 0.01$, compared with control values.

**Table 3** Effect of oral administration of St John's wort and SSRIs on $K_d$ and $B_{max}$ values for specific [$^3$H]paroxetine binding in mouse brain

| Drug | n | Time (h) | $K_d$ (nM) | $B_{max}$ (fmol (mg protein)$^{-1}$) |
|---|---|---|---|---|
| Control | 5 | | 0.13 ± 0.01 | 292 ± 5 |
| St John's wort 900 mg kg$^{-1}$ | 5 | 4 | 0.11 ± 0.01 | 272 ± 9 |
| Paroxetine 1 mg kg$^{-1}$ | 4 | 4 | 1.96 ± 0.26* | 204 ± 20* |
| Sertraline 10 mg kg$^{-1}$ | 4 | 4 | 2.07 ± 0.30* | 272 ± 43 |

Values are means ± s.e. of 4 or 5 mice. *$P < 0.05$, compared with control values.

was examined. Synaptosomal [$^3$H]serotonin uptake was little affected by oral administration of St John's wort, at a dose of 300 mg kg$^{-1}$ (Figure 1, Table 2) but was significantly suppressed by a dose of 900 mg kg$^{-1}$. In fact, the $K_m$ values were enhanced by 25.4 and 77.8%, respectively, at 1 and 4 h after the St John's wort (900 mg kg$^{-1}$) administration compared with control values, the value at 4 h being statistically significant, with little change in $V_{max}$ values. Similarly, paroxetine (1 mg kg$^{-1}$) and sertraline (10 mg kg$^{-1}$) brought about significant increase in $K_m$ values for [$^3$H]serotonin uptake 4 h after their oral administration, the increase being 6.38 and 7.46 fold for paroxetine and sertraline, respectively. A significant (24%) decrease in the $V_{max}$ value was also observed for sertraline.

Oral administration of paroxetine (1 mg kg$^{-1}$) and sertraline (10 mg kg$^{-1}$) produced significant (15.1 and 15.9 fold, respectively) increases in $K_d$ values for specific [$^3$H]paroxetine binding in mouse brain at 4 h compared with the control value, and also a significant (30%) decrease in the $B_{max}$ value (paroxetine) (Table 3). On the contrary, St John's wort (900 mg kg$^{-1}$) had little effect on $K_d$ and $B_{max}$ for brain [$^3$H]paroxetine binding.

## Effect of St John's wort and SSRIs on marble-burying behaviour and immobility time in the forced swimming test

Oral administration of St John's wort (300, 900 mg kg$^{-1}$) suppressed marble-burying behaviour in mice in a dose-dependent manner (Figure 2). The decrease in the number of marbles buried by mice at 4 h was 26.2% after a dose of 300 mg kg$^{-1}$; after 900 mg kg$^{-1}$ the decrease was 46.4, 95.2 and 29.8% at 1, 4 and 12 h, respectively (the decrease at 4 h

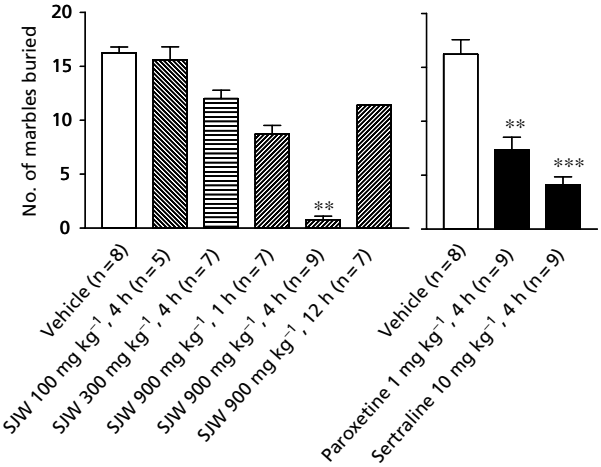


**Figure 2** Effect of oral administration of St John's wort (SJW), paroxetine and sertraline on marble-burying behaviour in mice. Mice received St John's wort (100, 300 or 900 mg kg$^{-1}$), paroxetine (1 mg kg$^{-1}$) or sertraline (10 mg kg$^{-1}$) orally, and they were subjected to the marble-burying test 1–12 h later. Mice were placed into the open cubic plastic box (22.5 × 33.8 × 14.0 cm) where 20 clean glass marbles (15 mm diameter) were evenly spaced (5 cm apart) on sawdust (5 cm deep), and the number of marbles buried by mice during the testing period (30 min) was counted. Each column represents the mean ± s.e. of 5–9 mice. $^{**}P < 0.01$, $^{***}P < 0.001$, compared with vehicle control values.

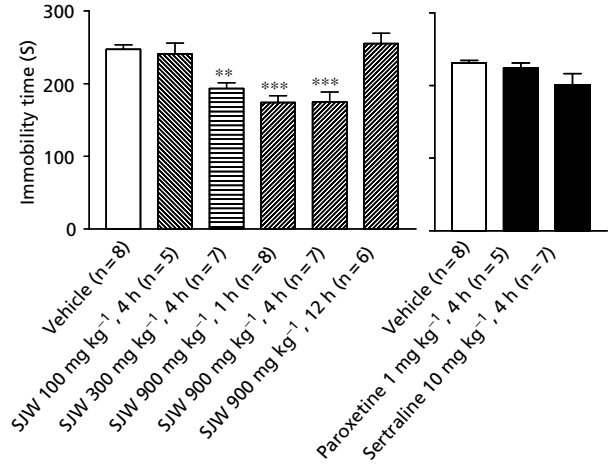


**Figure 3** Effect of oral administration of St John's wort (SJW), paroxetine and sertraline on the immobility time of mice in the forced swimming test. Mice received St John's wort (100, 300 or 900 mg kg$^{-1}$), paroxetine (1 mg kg$^{-1}$) or sertraline (10 mg kg$^{-1}$) orally, and they were subjected to the forced swimming test 1–12 h later. Mice were dropped individually into glass cylinders (height: 25 cm, diameter: 10 cm) containing 10 cm of water, and left there for 6 min. A mouse was judged to be immobile when it floated in an upright position, and made only small movements to keep its head above water. The total immobility time of each mouse was expressed in seconds as the sum of immobility periods during the 6-min forced swimming test. Each column represents mean ± s.e. of 5–8 mice. $^{**}P < 0.01$, $^{***}P < 0.001$, compared with vehicle control values.

after 900 mg kg$^{-1}$ was statistically significant). Similarly, paroxetine (1 mg kg$^{-1}$) and sertraline (10 mg kg$^{-1}$) significantly attenuated the marble-burying behaviour at 4 h after oral administration (54.6 and 74.5%, respectively).

In the forced swimming test, there was significant decrease in the immobility time of mice after oral administration of St John's wort and the reduction rates were 21.8% (300 mg kg$^{-1}$, 4 h), 29.6 and 29.2% (900 mg kg$^{-1}$, 1 and 4 h, respectively) (Figure 3). On the other hand, the immobility time of the mice was unaffected by oral administration of paroxetine and sertraline. Locomotor activity counts during the testing period (30 min) in mice remained unchanged after oral administration of each drug compared with the vehicle control group (Table 4).

**Table 4** Effect of oral administration of St John's wort and SSRIs on total activity in mice

| Drug | n | Time (h) | Total activity (counts) |
|---|---|---|---|
| Control | 7 | | 1160 ± 40 |
| St John's wort 100 mg kg$^{-1}$ | 5 | 4 | 1080 ± 40 |
| St John's wort 300 mg kg$^{-1}$ | 6 | 4 | 1210 ± 50 |
| St John's wort 900 mg kg$^{-1}$ | 6 | 1 | 1100 ± 90 |
| St John's wort 900 mg kg$^{-1}$ | 5 | 4 | 1090 ± 50 |
| St John's wort 900 mg kg$^{-1}$ | 5 | 12 | 1220 ± 70 |
| Paroxetine 1 mg kg$^{-1}$ | 7 | 4 | 1150 ± 50 |
| Sertraline 10 mg kg$^{-1}$ | 7 | 4 | 1160 ± 40 |

Values are means ± s.e. of 5–7 mice.

## Discussion

The relationship between brain serotonin transporter binding, effects on serotonin uptake and behaviour in mice was investigated after oral administration of St John's wort, in comparison with SSRIs, to elucidate the pharmacological relevance of in-vitro observation. In in-vitro experiments, fluvoxamine, fluoxetine, paroxetine and sertraline inhibited both [$^3$H]serotonin uptake and [$^3$H]paroxetine binding in mouse brain at a nanomolar range. The calculated IC50 values for [$^3$H]serotonin uptake and the $K_i$ values for [$^3$H]paroxetine binding of each SSRI became similar and there was a significant ($r = 0.98$, $P < 0.01$) correlation between both parameters. On the contrary, St John's wort inhibited [$^3$H]serotonin uptake into mouse brain synaptosomes in a concentration-dependent manner but had little effect on [$^3$H]paroxetine binding, which is consistent with previous reports (Gobbi et al 1999; Singer et al 1999). The IC50 value expressed as hyperforin equivalent of 3.2% extract became 179 nM and it was similar to the value for hyperforin (205 nM) reported previously (Chatterjee et al 1998). These data suggest that the inhibitory effect of St John's wort on serotonin uptake into brain synaptosomes is not due to direct interaction with [$^3$H]paroxetine binding sites

on serotonin transporters, and hyperforin plays a major role as an active ingredient of this extract in-vitro.

A series of bioactive compounds, such as phenylpropanes, flavonol derivatives, biflavones, proanthocyanidines, xanthones, phloroglucinols, some amino acids, naphthodianthrones and essential oil constituents, have been identified as constituents contained in St John's wort (Nahrstedt & Butterweck 1997) and extensive scientific studies have contributed towards the elucidation of their pharmacological effects and mode of action (Butterweck 2003). However, the effects and the mechanism of action of these constituents are still a matter of debate and the pharmacological effects of St John's wort can not be explained by a single compound such as hyperforin, as previously described (Butterweck 2003; Butterweck et al 2003). Thus, based on recent reports, it is likely that synergistic and antagonistic interaction among the various ingredients is important and multiple bioactive compounds contribute to the antidepressive effects of St John's wort in a complex manner. Therefore, in this study, the effects of systemic administration of St John's wort (whole extract) on brain serotonin transporters, serotonin uptake and behaviour in mice were investigated, since the situation under the influence of pharmacokinetic and pharmacodynamic factors makes it possible to take not only its constituents but also its metabolites into consideration.

Oral administration of St John's wort ($900\,mg\,kg^{-1}$), paroxetine ($1\,mg\,kg^{-1}$) and sertraline ($10\,mg\,kg^{-1}$) produced a significant increase in $K_m$ value for [$^3$H]serotonin uptake into mouse brain synaptosomes after 4 h. Paroxetine and sertraline significantly suppressed specific [$^3$H]paroxetine binding in mouse brain as revealed by a significant increase in $K_d$, whereas St John's wort had little effect on binding parameters for [$^3$H]paroxetine. Thus, in accordance with in-vitro experiments, our data suggest that SSRIs, but not St John's wort, suppress serotonin uptake into brain synaptosomes through binding to serotonin transporters and that none of the constituents or metabolites have a significant affinity for serotonin transporters in mouse brain after oral administration of St John's wort. Systemic administration of St John's wort ($125–500\,mg\,kg^{-1}$, p.o.) significantly increased serotonin levels in the cortex of rats and noradrenaline and dopamine levels in the diencephalon (Calapai et al 1999), and hyperforin alone ($10\,mg\,kg^{-1}$, i.p.) produced a significant increase in extracellular serotonin, noradrenaline, dopamine and glutamate in the rat locus coeruleus using microdialysis (Kaehler et al 1999). Nonselective inhibition by St John's wort of various neurotransmitter uptake into presynaptic neurons has been reported. According to the in-vitro observation, the mechanism underlying this inhibitory effect might be due to an increase in free intracellular sodium concentration by hyperforin (Singer et al 1999) through interaction with amiloride-sensitive sodium-conductive pathways such as the $Na^+$ channel and $Na^+$–$H^+$ exchanger (Wonnemann et al 2000). It is well known that the sodium gradient is the driving force of all neurotransmitter transporters (Lester et al 1994) and this property would result in nonselective uptake inhibition by St John's wort. Such nonspecific uptake inhibition of St John's wort, which may be one of the possible mechanisms contributing to the antidepressant effect, is supported by our observation that this extract inhibited synaptosomal uptake of serotonin without interacting with serotonin transporters.

The marble-burying test and forced swimming test are utilized to evaluate the therapeutic effects in obsessive–compulsive disorder and depression, respectively (Porsolt et al 1978; Njung'e & Handley 1991; Ichimaru et al 1995) and we investigated the potential behavioural activity of St John's wort in comparison with SSRIs. Oral administration of St John's wort ($900\,mg\,kg^{-1}$) significantly suppressed marble-burying behaviour in mice, with little change in locomotor activity, and the time-course of the suppression of this behaviour approximately paralleled that of [$^3$H]serotonin uptake inhibition. Similarly, significant attenuation of this behaviour was observed after oral treatment with paroxetine ($1\,mg\,kg^{-1}$) and sertraline ($10\,mg\,kg^{-1}$). In accordance with our results, the suppressive effect of St John's wort ($300\,mg\,kg^{-1}$, p.o.) on marble-burying behaviour in mice was reported by Skalisz et al (2004). We have previously found that brain serotonin transporter occupancy by SSRIs significantly correlated well with their suppressive effects on marble-burying behaviour after oral administration in mice (unpublished observation), suggesting that St John's wort exerts its suppressive effect on marble-burying behaviour through the activation of serotonergic neurotransmission. In the forced swimming test, St John's wort, but not SSRIs, significantly decreased the immobility time of mice without changing locomotor activity. The potential antidepressant activity of St John's wort in the forced swimming test is well known (Chatterjee et al 1998; Butterweck et al 2003) and the lack of efficacy of SSRIs on this behavioural model at the doses used here is also consistent with previous reports (Cervo et al 1991; Sánchez & Meier 1997). Systemic treatment with paroxetine ($3\,mg\,kg^{-1}$, s.c.) and sertraline ($10\,mg\,kg^{-1}$, s.c.) selectively increased the brain level of extracellular serotonin but not noradrenaline and dopamine (Bymaster et al 2002), and imipramine and desipramine, which have a high affinity for noradrenaline transporters, are utilized as positive controls in the forced swimming test (Egawa et al 1995; Sánchez & Meier 1997). Since St John's wort significantly suppressed both the marble-burying behaviour and the immobility time in the forced swimming test, it is plausible that St John's wort administered systemically enhances not only serotonergic neurotransmission but also noradrenergic neurotransmission to pharmacologically effective levels. Also, the involvement of other neurotransmitter pathways cannot be excluded (Calapai et al 1999; Kaehler et al 1999).

## Conclusions

This study has shown that oral administration of St John's wort inhibits brain serotonin uptake without interacting with the transporter molecule. Furthermore, this extract effectively suppressed both the marble-burying behaviour and the immobility time in the forced swimming test in mice. These results provide the first in-vivo evidence to suggest that the mode of antidepressant action of St John's wort differs from that of SSRIs. Thus, this study may have a significant impact on phytotherapy with St John's wort.

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