Hyperforin, a Major Antidepressant Constituent of St. John’s Wort, Inhibits Serotonin Uptake by Elevating Free Intracellular Na\(^{+}\)\(^{1}\)

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ABSTRACT

Extracts of Hypericum perforatum (St. John’s Wort) are widely used for the treatment of depressive disorders and are unspecific inhibitors of the neuronal uptake of several neurotransmitters. Previous studies have shown that hyperforin represents the reuptake inhibiting constituent. To characterize the mechanism of serotonin reuptake inhibition, kinetic analyses in synaptosomes of mouse brain were performed. Michaelis-Menten kinetics revealed that hyperforin (2 \(\mu\)M) induces a decrease in \(V_{\text{max}}\) by more than 50% while only slightly decreasing \(K_{\text{m}}\), indicating mainly noncompetitive inhibition. By contrast, citalopram (1 \(\text{nM}\)) leads to an elevation of \(K_{\text{m}}\) without changing \(V_{\text{max}}\). Monensin, a Na\(^+\)/H\(^+\) exchanger, showed similar properties as hyperforin (decrease of \(V_{\text{max}}\) without changing \(K_{\text{m}}\)). Compared with classical antidepressants, such as selective serotonin reuptake inhibitors and tricyclic antidepressants, hyperforin is only a weak inhibitor of \([\text{H}]\text{paroxetine binding relative to its effects on serotonin uptake. As monensin decreases serotonin uptake by increasing Na}\(^+\)/H\(^+\) exchange, we compared the effects of hyperforin and monensin on the free intracellular sodium concentration ([Na\(^+\)]) in platelets by measuring 1,3-benzenedicarboxylic acid, 4,4′-[1,4,10-trioxa-7,13-diazacyclopentadecan-7,13-diylbis(5-methoxy-6,2-benzofurandiy)]bis-, tetraammonium salt (SBFI/AM) fluorescence. Both drugs elevated [Na\(^+\)] over basal levels, with a maximal [Na\(^+\)], of 69 ± 16.1 \(\mu\)M (50 \(\mu\)M hyperforin) and 140 ± 9.1 \(\mu\)M (10 \(\mu\)M monensin). Citalopram at concentrations relevant for \([\text{H}]\text{serotonin uptake inhibition had no effect on [Na}\(^+\)]. Although the mode of action of hyperforin seems to be associated with elevated [Na\(^+\)], similar to those levels found with monensin, the molecular mechanism might be different, as even at high concentrations, hyperforin does not elevate free intracellular sodium concentration ([Na\(^+\)]) up to the extracellular level, as monensin does. Hyperforin represents the first substance with a known preclinical antidepressant profile that inhibits serotonin uptake by elevating [Na\(^+\)].

Extracts of the medicinal plant Hypericum perforatum (St. John’s Wort) are broadly used in many countries to treat depressive disorders. Several recent reviews of controlled clinical studies with hypericum extract have come to the conclusion that it represents an effective antidepressant principle superior to placebo (Linde et al., 1996; Volz, 1997; Wheatley, 1998; Wong et al., 1998). In most clinical studies, hypericum extract was used at a dosage of 300 mg three times daily. A favorable side effect profile is considered its most relevant advantage. In agreement with the possible therapeutic potential, many recent pharmacological studies with hypericum extract also support its antidepressant activity. The extract inhibits the synaptosomal uptake of noradrenaline, serotonin, and dopamine, induces \(\beta\)-receptor down-regulation when given chronically to rats, and is active in a large variety of behavioral models indicative of antidepressant activity (Bhattacharya et al., 1998; Butterweck et al., 1997; Chatterjee et al., 1998; Müller et al., 1998). However, monoamine oxidase (MAO)-A- and MAO-B-inhibiting properties of the extract are probably too weak to significantly contribute to its antidepressant activity (Cott, 1997; Müller et al., 1997).

The extract contains a large number of constituents. It is not known which of these constituents is responsible for the antidepressant properties of the extract. The original assumption (Suzuki et al., 1984), that the naphthodianthrone derivative hypericin is specifically relevant by inhibiting MAO-A, has not been confirmed by several subsequent studies (Cott, 1997; Müller et al., 1997). Recently, the phloroglucinol derivative hypericin became of increasing interest, as

ABBREVIATIONS: MAO, monoamine oxidase; SBFI/AM, 1,3-benzenedicarboxylic acid, 4,4′-[1,4,10-trioxa-7,13-diazacyclopentadecan-7,13-diylbis(5-methoxy-6,2-benzofurandiy)]bis-, tetraammonium salt; RT, room temperature; SSRI, selective serotonin reuptake inhibitor; [Na\(^+\)], free intracellular sodium concentration.
it represents the major reuptake-inhibiting component of hypericum extract (Chatterjee et al., 1998; Muller et al., 1998). Moreover, it leads to elevated extracellular brain concentrations of serotonin, norepinephrine, and dopamine after administration to rats (Kaehler et al., 1999), down-regulates beta-receptors (Muller et al., 1997), is active in several behavioral models indicative of antidepressant activity (Chatterjee et al., 1998; Bhattacharya et al., 1998), leads to specific changes of the rat and human EEG typical for selective serotonin reuptake inhibitors (SSRIs; Dimpfel et al., 1998; Schellenberg et al., 1998), and reaches plasma levels in the rat needed to inhibit synaptosomal uptake of several neurotransmitters (about 700 nM; Biber et al., 1998) at a single dose of hypericum extract of 300 mg/kg, which is effective in most behavioral studies (Bhattacharya et al., 1998; Butterweck et al., 1998). In humans, hyperforin plasma levels of about 600 nM were seen after a single dose of 600 mg of hypericum extract (Biber et al., 1998). It also seems to be relevant for the antidepressant activity of hypericum extract in humans (Laakmann et al., 1998). Thus, hyperforin has to be considered an important antidepressant constituent of hypericum extract.

Contrary to all other antidepressant drugs known, hyperforin not only potently inhibits the synaptosomal uptake of norepinephrine, dopamine, and serotonin, but also the uptake of the two amino acid transmitters, L-aminobutyric acid and L-glutamate (Chatterjee et al., 1998). This might point to a different mechanism of action, probably not associated with specific binding sites at the transporter molecules. Accordingly, we have performed several additional experiments comparing the molecular basis of the serotonin uptake inhibition by hyperforin with that of SSRIs like citalopram and with the uptake inhibition by the sodium ionophore monensin (Reith and O'Reilly, 1990; Izenwasser et al., 1992). Monensin has been shown to elevate [Na\(^+\)]\(_i\) in a variety of different cell types in vitro (Izenwasser et al., 1992; Adovedlande and Schrevé, 1996; Rochdi et al., 1996). It has been used for many years in veterinary medicine to treat gastrointestinal infections like coccidiosis in cattle (Bergen and Bates, 1984). As it unspecifically enhances intracellular sodium concentration, it is rather toxic. Monensin is a very hydrophilic molecule and is probably not reaching the brain.

In the present study, it was exclusively used as a tool for in vitro experiments to elevate free intracellular sodium by an unspecific physicochemical mechanism independent of the physiological systems regulating sodium conductance.

### Experimental Procedures

#### Synaptosomal Uptake of Serotonin

Synaptosomal preparations from the frontal cortex of female NMRI mice were used for serotonin uptake. The tissue was homogenized in ice-cold sucrose solution (0.32 M) and diluted with 10 ml of the homogenizing medium. The nuclear fraction was eliminated by centrifugation at 750g for 10 min (Beckman Centrifuge, Model J2–21; Beckman Coulter, Fullerton, CA). The supernatant was centrifuged at 17400g for 20 min to obtain the crude synaptosomal pellets. The pellets were suspended in 11 ml of ice-cold Krebs-HEPES-buffer (150 mM NaCl, 10 mM HEPES, 6.2 mM KCl, 1.2 mM Na\(_2\)HPO\(_4\), 1.2 mM MgSO\(_4\), 10 mM Glucose, 10 \(\mu\)M Pargylin, and 0.1% ascorbic acid; pH 7.4 at 37°C), aliquoted in microtiter plates, incubated in the presence of varying concentrations of the drugs tested at 37°C for 15 min in a shaking water bath, and cooled on ice. The \(^3\)H-labeled tracer (2.9 nM serotonin) was added and the uptake started by incubation at 37°C for 4 min, during which time uptake is linearly dependent on time. The probes were cooled, immediately filtered through Whatman GF/B glass fiber filters, and washed three times with ice-cold buffer solution with a Brandel cell harvester. Filters were placed in plastic scintillation vials containing 4 ml of Lumasafe scintillation cocktail (Packard, Meriden, CT). Radioactivity was measured after 12 h. Nonspecific uptake was determined in parallel probes maintained throughout on ice or containing unlabeled serotonin (1 mM serotonin).

#### Kinetic Analyses

The transport of \(^3\)H-serotonin into synaptosomal preparations of the frontal cortex of mice was measured by the same method mentioned above in the presence of varying concentrations of \(^3\)H-serotonin (1–30 nM). Kinetic constants for uptake were obtained by direct weighted nonlinear regression of active uptake against \(^3\)H-serotonin concentration with GraphPad PRISM software (GraphPad, San Diego, CA).

\[^3\]H\)Serotonin Uptake in Platelets

Blood from normal unmedicated volunteers was anticoagulated in S-Monovettes with 1 ml of citrate solution and centrifuged for 15 min at 250g at room temperature. The upper two-thirds of the platelet-rich plasma was collected in a Falcon tube and centrifuged at 800g for 30 min at room temperature (RT). The resulting pellet was resuspended in a 4-fold amount of Krebs-Henseleit phosphate buffer (KHP buffer; 6.92 g of NaCl, 0.35 g of KCl, 0.29 g of MgSO\(_4\), 0.16 g of KH\(_2\)PO\(_4\), 2.1 g of glucose, and 1 g of ascorbic acid per l; pH 7.4 at 37°C). Platelets were preincubated in a shaking water bath for 15 min with the various agents before the addition of \(^3\)H-serotonin (2.9 nM). The incubation was continued for 2 min after the addition of \(^3\)H-serotonin. After this time, the uptake was terminated by dilution of the samples with ice-cold buffer and rapid filtration through Whatman GF/C glass fiber filters. The microtiter plates and the filters were washed three times with a Brandel cell harvester (Bran del, Bethesda, MD). The filters were dried and radioactivity was determined by liquid scintillation counting. Each point was determined in triplicate. Nonspecific uptake was determined in parallel experiments containing 1 mM cold serotonin.

\[^3\]H\)Paroxetine Binding Assay

Female NMRI mice were sacrificed by decapitation. The frontal cortex was homogenized in 40 volumes of TRIS buffer (50 mM TRIS HCl, 120 mM NaCl, and 5 mM KCl; pH 7.4 at 0°C) in a glass Potter-Elvehjem homogenizer with Teflon pestle. The homogenate was centrifuged for 10 min at 35000g at 4°C. The resulting pellet was resuspended in 40 volumes of buffer and recentrifuged. The pellet was resuspended in TRIS buffer at a protein concentration of 70 \(\mu\)g/ml. Aliquots of membrane suspension were incubated with \[^3\]H\)paroxetine (0.2 nM) at room temperature for 60 min. Incubation was terminated by rapid filtration through Whatman GF/B glass fiber filters with a Brandel Cell Harvester. Filters were routinely pretreated with 0.025% Brij 35 (ICI, London, United Kingdom). The filters were washed seven times with ice-cold buffer and dried, and the radioactivity was measured by liquid scintillation spectrometry. Specific binding of \[^3\]H\)paroxetine was defined as the difference between the total binding and that remaining in the presence of 10 \(\mu\)M fluoxetine.

### Measuring \([\text{Na}^+]_i\) with 1,3-Benzeneedicarboxylic Acid, 4,4'-[1,4,10-Trioxa-7,13-diazacyclopentadecan-7,13-diylbis[5-methoxy-6,2-benzofurandiyl]]bis-, Tetraammonium Salt (SBFI/AM)

#### Solutions

- Solutions. The Na\(^+\)–HEPES buffer consisted of 145 mM NaCl, 10 mM HEPES, 1 mM MgSO\(_4\), 5 mM KH\(_2\)PO\(_4\), 5 mM KCl, and 5 mM glucose (Borin and Siffert, 1990). The pH of this buffer was adjusted
to 6.5 with NH₃ for the storage of platelets and to 7.4 for the measurement of fluorescence. The K⁺ solution was identical except for complete replacement of sodium by potassium. By mixing these solutions together, any final sodium concentration between 0 and 145 mM can be obtained.

Preparation of Platelets and Loading with SBFI/AM. Venous blood (10 ml) was anticoagulated by mixing it with 1 ml of citrate solution in a S-Monovette. The anticoagulated blood was centrifuged at 250g for 15 min at room temperature. The upper two-thirds of the supernatant was gently removed with a plastic Pasteur pipette. Platelet pellets were obtained from platelet-rich plasma by centrifugation at 480g for 30 min at room temperature. The pellet was resuspended into sodium-HEPES buffer (pH 6.5) at a final concentration of 3 to 4 × 10⁸ cells/ml. Immediately before addition to the platelets, 1 volume of SBFI/AM solution was mixed with 2 volumes of the nonionic detergent Pluronic F-127. Appropriate amounts of this solution were added to the platelets to yield a final concentration of SBFI/AM of 10 µM, and platelets were incubated with the dye for 60 min at 37°C in a shaking water bath. Thereafter, platelets were again centrifuged at 480g for 30 min at RT. The supernatant was discarded and the platelets were taken up into sodium-HEPES buffer (pH 6.5) at a concentration of 3 to 4 × 10⁹ cells/ml. The platelet suspension was then stored at RT until it was used for the fluorescence measurements.

Measurement of SBFI/AM Fluorescence. Aliquots of platelet suspension (4 µl) were added to 950 µl of the appropriate buffer. Fluorescence was measured in quartz cuvettes with an Aminco-Bowman Series 2 (SLM-Aminco, Rochester, NY) luminescence spectrometer before and after the addition of the tested drugs. Samples were excited alternately at 345 and 385 nm (bandwidth = 5 nm) and fluorescence was recorded at 490 nm (bandwidth = 16 nm). Fluorescence excitation ratios of 345/385 nm were calculated. All measurements were performed at room temperature without stirring.

Calibration of SBFI/AM fluorescence in terms of [Na⁺]ᵢ, was performed according to the method of Harootunian et al. (1989). By the addition of platelets to solutions of known extracellular sodium concentrations, which were made by mixing different amounts of Na⁺-HEPES and K⁺-HEPES buffer, the 345/385 nm intensity ratio was determined before and 5 min after the addition of the ionophore gramicidin D (2 µM final concentration). Baseline [Na⁺]ᵢ (mean of 15 experiments ± S.D.) was 32 mM ± 6, which is in agreement with the data by Borin and Siffert (1990).

Determination of Protein Concentration. Protein concentration was determined according to the method of Bradford (1976) with the Bio-Rad Protein Assay Kit.

Materials
Standard laboratory chemicals were obtained from Sigma-Aldrich Chemical (Deisenhofen, Germany) or were a gift from Merck (Darmstadt, Germany). SBFI/AM and gramicidin D were purchased from Molecular Probes (Eugene, OR). [³H]serotonin and [³H]paroxetine were obtained from New England Nuclear Life Science Products (Boston, MA). Brij 35 was obtained from Sigma (Munich, Germany), and Pluronic F-127 and monensin were obtained from Calbiochem (Frankfurt, Germany). Citalopram was a gift from Lundbeck (Hamburg, Germany). Hyperforin was generously supplied by Schwabe (Karlsruhe, Germany). Fluoxetine was purchased from Tocris (Bristol, UK).

Results
Figure 1 shows the concentration-dependent inhibition by hyperforin of [³H]serotonin uptake into mouse brain synaptosomes. A monophasic effect with a Hill coefficient of about 1 was found, which is in agreement with previous findings (Chatterjee et al., 1998; Müller et al., 1998).

Binding of [³H]paroxetine to brain membranes labels the serotonin binding site of the human and rodent serotonin transporter molecule (Marcusson and Ross, 1990; Gurevich and Joyce, 1996). This is supported by our findings showing a good correlation between the IC₅₀ values for inhibition of [³H]paroxetine binding and for inhibition of [³H]serotonin uptake (Fig. 2). For the antidepressant drugs investigated, ratios varied between 1 and 3 (Table 1). However, the inhibitory effect of hyperforin on [³H]serotonin uptake is much stronger than its potency to inhibit [³H]paroxetine binding (R = 14; Table 1).

Our kinetic analyses (Table 2) indicate that hyperforin (2 µM) decreases the Vₘₐₓ of synaptosomal serotonin uptake from 0.191 ± 0.034 to 0.083 ± 0.032 pmol/min/mg of protein. By contrast, hyperforin did not increase the Kₘ (Table 2), but slightly decreased Kₘ. The findings suggest that inhibition by this substance is mainly noncompetitive. In comparison, the SSRI citalopram (1 nM) leads to an increase in Kₘ without affecting the Vₘₐₓ value, confirming previous findings indicative of competitive inhibition of [³H]serotonin uptake (Hyttel, 1978).

These findings do not support the assumption that hyper-
IC₅₀ for specific [³H]serotonin uptake and for specific [³H]paroxetine binding. Data are means ± S.D. of six experiments.

<table>
<thead>
<tr>
<th>Substance</th>
<th>[³H]Paroxetine Binding</th>
<th>[³H]Serotonin Uptake</th>
<th>Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clomipramine</td>
<td>1.9 ± 0.4</td>
<td>1.0 ± 0.1</td>
<td>1.9</td>
</tr>
<tr>
<td>Sertraline</td>
<td>3.3 ± 0.3</td>
<td>2.1 ± 1.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Citalopram</td>
<td>4.1 ± 0.6</td>
<td>1.1 ± 0.2</td>
<td>3.7</td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>13.5 ± 3.7</td>
<td>10.9 ± 5.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>26.9 ± 0.6</td>
<td>10.4 ± 5.1</td>
<td>2.6</td>
</tr>
<tr>
<td>Monensin</td>
<td>47.000</td>
<td>47.8 ± 16.3</td>
<td></td>
</tr>
<tr>
<td>Désipramine</td>
<td>672.3 ± 171.6</td>
<td>214.3 ± 89.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Hyperforin</td>
<td>19,528 ± 6,983</td>
<td>1,357 ± 171</td>
<td>14.4</td>
</tr>
</tbody>
</table>

* IC₅₀ [³H]paroxetine binding/IC₅₀ [³H]serotonin uptake.

**TABLE 2**

Kinetic analyses of the effects of citalopram, hyperforin, and monensin on [³H]serotonin uptake into mouse cortex synaptosomes. Data are means ± S.D. of six determinations.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Kₘ (nM)</th>
<th>Vₘₐₓ (pmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.72 ± 5.01</td>
<td>0.105 ± 0.044</td>
</tr>
<tr>
<td>Citalopram (1 nM)</td>
<td>29.24 ± 5.25**</td>
<td>0.106 ± 0.074</td>
</tr>
<tr>
<td>Control</td>
<td>11.90 ± 2.07</td>
<td>0.191 ± 0.034</td>
</tr>
<tr>
<td>Hyperforin (2 µM)</td>
<td>9.93 ± 0.59*</td>
<td>0.063 ± 0.032**</td>
</tr>
<tr>
<td>Control</td>
<td>13.78 ± 2.50</td>
<td>0.132 ± 0.039</td>
</tr>
<tr>
<td>Monensin (100 nM)</td>
<td>14.72 ± 3.94</td>
<td>0.065 ± 0.009**</td>
</tr>
</tbody>
</table>

*p < .05.
**p < .001 using unpaired t-test.

Monensin competitively binds to the serotonin binding site of the transporter molecule, as is the case with most antidepressant drugs. Therefore, we investigated other parameters known to affect [³H]serotonin uptake. It is generally accepted that serotonin uptake requires external sodium (Sneddon, 1969, 1973; Bogdanski et al., 1979) and can be inhibited by the sodium ionophore monensin (Reith and O’Reilly, 1990; Izenwasser et al., 1992). The potent inhibition of synaptosomal uptake by monensin could be confirmed (Table 1; Fig. 1). Similar to hyperforin, a Hill coefficient of about 1 was observed (Fig. 1). Moreover, monensin also represents a non-competitive inhibitor: it has no effect on Kₘ but decreases Vₘₐₓ more than 50% (Table 2) and has no affinity for the transporter binding site labeled with [³H]paroxetine (Table 1).

To investigate the possible relevance of the sodium gradient for the uptake inhibition, we examined the effect of monensin on the free intracellular Na⁺ concentration with the Na⁺ indicator SBFI/AM. As this method requires incubation at 37°C for an extended period, the experiments were performed with human platelets. Platelets repeatedly have been used as a model system for the serotonin transport into neurons (Sneddon, 1973). Comparison of [³H]serotonin uptake inhibition in mouse brain synaptosomes and human platelets shows comparable IC₅₀ values for hyperforin, monensin, and two antidepressants drugs in both systems (Table 3). This not only indicates similar properties of serotonin uptake into both cell systems, but also demonstrates for the first time the efficacy of hyperforin in a cell model expressing the human serotonin transporter.

The sodium ionophore monensin has been shown to induce sodium-proton exchange across the cellular membrane (Sandeaux et al., 1982; Riddell et al., 1988; Katsuyoshi and Yoshikawa, 1991). This could be confirmed for human platelets, where monensin elevated intracellular Na⁺ concentrations in a time- and concentration-dependent fashion (Fig. 3). Equilibration was obtained after 15 min of incubation. Maximal effects were seen in the presence of high concentrations of monensin leading to an elevation of [Na⁺]ᵢ to the extracellular level (Fig. 4, top). There was a close relationship between the effects of monensin on [Na⁺]ᵢ and on serotonin uptake, supporting the notion that this ionophore inhibits serotonin uptake by elevating [Na⁺]ᵢ (Fig. 4, top). At about 80% inhibition of uptake, [Na⁺]ᵢ was elevated by about 20 mM (Fig. 4, bottom). When investigated under similar con-
ditions, hyperforin also increased $[\text{Na}^+]_i$ in human platelets at the same concentrations needed to inhibit serotonin uptake (Fig. 5, top). Similar to monensin, the effect of hyperforin was maximal within 15 min of incubation (Fig. 3). In contrast to monensin, hyperforin showed a reverse U-shaped dose-response curve. Maximal elevation of $[\text{Na}^+]_i$ by about 70 mM was seen at 50 $\mu$M hyperforin. Higher concentrations of hyperforin had no further effects (Fig. 5, top) or were even significantly less active (100 $\mu$M hyperforin; $p < .05$ versus 50 $\mu$M; Fig. 5, top). In contrast, when investigated under similar conditions, citalopram (1–10,000 nM) did not alter $[\text{Na}^+]_i$ in human platelets (data not shown).

**Discussion**

Our findings with mouse brain synaptosomes and human platelets demonstrate that hyperforin, like many other antidepressant drugs, inhibits serotonin uptake in both systems with similar potencies. As far as we know, this is the first evidence that hyperforin is also active at the human serotonin transporter. However, the rather weak binding to the serotonin transporter labeled by $[^3\text{H}] \text{paroxetine}$ binding in comparison to the much stronger effect on serotonin uptake might indicate that the binding to the transporter site labeled by $[^3\text{H}] \text{paroxetine}$ is not the primary mechanism responsible for the uptake inhibition. The assumption is further supported by our observation of a noncompetitive type of inhibition by hyperforin, contrary to the competitive type of inhibition seen for citalopram. Moreover, hyperforin is a rather nonselective synaptosomal uptake inhibitor (Chattjee et al., 1998; Müller et al., 1998), which also argues against a specific binding site as the primary mechanism of action.

Thus, we speculated that hyperforin works by elevating $[\text{Na}^+]_i$. Because the sodium cotransport into the cell represents the driving force of the serotonin transporter, the serotonin uptake system is very sensitive to conditions leading to reduced extracellular or enhanced intracellular sodium concentrations (Sneddon, 1969; Bogdanski et al., 1979).

An already known example of sodium dependency is the sodium ionophore monensin, which elevates $[\text{Na}^+]_i$ by promoting $\text{Na}^+/\text{H}^+$ exchange (Sandeaux et al., 1982; Riddell et al., 1988; Katsuoshi and Yoshihiko, 1991). We could confirm previous findings that monensin dose dependently inhibits $[^3\text{H}] \text{serotonin uptake into brain synaptosomes}$ (Reith and O'Reilly, 1990; Izenwasser et al., 1992). Inhibition into synaptosomes was noncompetitive. Similar to hyperforin, monensin inhibits the synaptosomal uptake of norepinephrine, dopamine, $\gamma$-aminobutyric acid, and $\alpha$-glutamate with IC$_{50}$ values in the nanomolar range (unpublished observations from our laboratory). Thus, monensin effects on synaptoso-
mal uptake systems show considerable similarities to the properties of hyperforin.

However, it was not known to what extent the inhibition of serotonin uptake by monensin is associated with an increase of [Na\(^+\)]. Therefore, we compared the effects of increasing concentrations of monensin on both parameters in human platelets, which are well characterized cell systems for both types of experiments. As expected, monensin at concentrations sufficient to inhibit \(^{[3]H}\)serotonin uptake led to a pronounced increase of [Na\(^+\)]. On the other hand, these findings clearly indicate that only rather small changes of [Na\(^+\)] are obviously sufficient to inhibit the serotonin transporter. At an inhibition of about 80% (500 nM monensin) [Na\(^+\)] was elevated over baseline levels by about 20 mM [Na\(^+\)]. In contrast, at concentrations relevant for serotonin uptake inhibition, citalopram had no effect at all on [Na\(^+\)], in human platelets. The effect of hyperforin was also associated with elevated [Na\(^+\)]. At a hyperforin concentration leading to about 80% uptake inhibition, an elevation of [Na\(^+\)] by about 20 mM again was found.

Our finding that hyperforin leads to an elevation of [Na\(^+\)], not only explains its effects on serotonin uptake into platelets and synaptosomes, but also explains its rather nonselектив profile on many neurotransmitter transport systems, all of which are driven by the Na\(^+\) gradient across the membrane (Lester et al., 1994). Although in many ways hyperforin resembles the ionophore monensin, it shows a reverse U-shaped dose-response curve and does not elevate [Na\(^+\)], up to the extracellular level as monensin does. Therefore, we speculate that hyperforin is not simply a sodium ionophore, but that its effect on [Na\(^+\)], is associated with one of the cellular ion-exchanging mechanisms. This could explain that its effects on [Na\(^+\)], are terminated once a certain level of [Na\(^+\)], is reached. Hyperforin represents the first substance known with a possible preclinical antidepressant profile that might work by influencing [Na\(^+\)]. A further characterization of the molecular basis of this possible new principle is currently under way.

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