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Inhibition of Synaptosomal Uptake of ^{3}H-L-glutamate and ^{3}H-GABA by Hyperforin, a Major Constituent of St. John's Wort: The Role of Amiloride Sensitive Sodium Conductive Pathways

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Extracts of St. John's Wort are widely used for the treatment of depressive disorders. The active principles have not yet been finally elucidated. We have recently shown that hyperforin, a major active constituent of St. John's Wort, not only inhibits the neuronal uptake of serotonin, norepinephrine and dopamine, but also that of L-glutamate and GABA. No other antidepressant compound exhibits a similar broad uptake inhibiting profile. To investigate this unique kind of property, kinetic analyses were performed regarding the uptake of ^{3}H-L-glutamate and ^{3}H-GABA into synaptosomal preparations of mouse brain. Michaelis-Menten kinetics revealed a reduction of V_{max} (8.27 to 1.80 pmol/mg/min for ^{3}H-L-glutamate, 2.76 to 0.77 pmol/mg/min for ^{3}H-GABA) while K_m was nearly unchanged in both cases, suggesting non-competitive inhibition. The unselective uptake inhibition by hyperforin could be mimicked by the Na^+- ionophore monensin and by the Na^+-K^+-ATPase inhibitor ouabain. However, both mechanisms can be discarded for hyperforin. Several amiloride derivatives known to affect sodium conductance significantly enhance ^{3}H-GABA and ^{3}H-L-glutamate uptake and inhibit the uptake inhibition by hyperforin, while monensin or ouabain inhibition were not influenced. Selective concentrations of benzamil for amiloride sensitive Na^+-channels and selective concentrations of 5′-ethylisopropylamiloride (EIPA) for the Na^+-H^+-exchangers both had an attenuating effect on the hyperforin inhibition of L-glutamate uptake, suggesting a possible role of amiloride sensitive Na^+-channels and Na^+-H^+-exchangers in the mechanism of action of hyperforin. ***[Neuropsychopharmacology 23:188–197, 2000]*** *© 2000 American College of Neuropsychopharmacology. Published by Elsevier Science Inc. All rights reserved*

KEY WORDS: *Hyperforin; St. John's Wort; Amiloride sensitive sodium channel; Na^+-H^+ exchange; Synaptosomal uptake inhibition*

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Received August 24, 1999; revised February 9, 2000; accepted February 14, 2000.

Several recent reviews of controlled clinical studies with St. John's Wort (hypericum) extract come to the conclusion that it represents an effective antidepressant treatment superior to placebo (Linde et al. 1996; Volz 1997; Wheatley 1998; Wong et al. 1998). In agreement with its clinical efficacy, hypericum extract is also active in a large number of biochemical and behavioral models which are indicative of antidepressant activity (Butterweck et al. 1997; Müller et al. 1997; Bhattacharya et al. 1998; Chatterjee et al. 1998a, 1998b; Gambarana et al. 1999). As possible mechanism of action an inhibition

0893-133X/00/$–see front matter
PII S0893-133X(00)00102-0

of the neuronal uptake of serotonin, norepinephrine and dopamine has been demonstrated (Müller et al. 1997; Neary and Bu 1999).

The lipophilic phloroglucinol derivative hyperforin was recently identified as a major active component of hypericum extract (Chatterjee et al. 1998a; Müller et al. 1998; Laakmann et al. 1998). It is a potent inhibitor of the uptake of serotonin, norepinephrine and dopamine, it is active in several biochemical and behavioral models of antidepressant activity (Bhattacharya et al. 1998; Chatterjee et al. 1998a, 1998b; Müller et al. 1997), it is responsible for specific changes of the rat and human EEG typically seen for specific serotonin reuptake inhibitors (Dimpfel et al. 1998; Schellenberg et al. 1998) and it elevates extracellular concentrations of serotonin, norepinephrine, and dopamine in the rat brain after i.p. administration (Kaehler et al. 1999). In addition to this rather typical antidepressant profile, hyperforin also potently inhibits the synaptosomal uptake of L-glutamate and GABA (Müller et al. 1998; Chatterjee et al. 1998a) and also enhances extracellular L-glutamate levels in rat brain (Kaehler et al. 1999). Since this property is unique among all other antidepressant compounds known, it was investigated in further detail. We have previously shown that inhibition by hyperforin of serotonin uptake into human platelets was associated with elevated free intracellular sodium concentration (Singer et al. 1999). As a similar effect could very likely explain the rather broad inhibiting properties of hyperforin for several synaptosomal uptake systems, we specifically investigated the possible role of amiloride sensitive sodium conductive pathways for the effects of this compound on ^{3}H-L-glutamate and ^{3}H-GABA uptake. A preliminary report of the data was previously published (Wonnemann et al. 1999).

METHODS

Animals

Female NMRI (Naval medical research institute, NIH Bethesda, USA) mice (2–3 months) were used for the uptake assays and were obtained from Harlan Winkelmann (Borchen, Germany). All animals were housed in plastic cages with water and food *ad libitum* and were maintained on a 12-hour light/dark cycle. All experiments were performed in accordance with the German animal right regulations.

Materials

Hyperforin was isolated from hypericum extract according to Erdelmeier (1998) and was a gift by Dr. Willmar Schwabe GmbH & Co (Karlsruhe, Germany). The hyperforin samples were stored in the dark at −20°C in nitrogen atmosphere. Tritiated radiochemicals (Glutamic acid L-^{3}H(G): spec.activity 1.11 TBq/mmol; Aminobutyric Acid γ [2,3-^{3}H]: spec. activity 1.11 TBq/mmol) were purchased from NEN Life science products (Dreieich, Germany) or Biotrend (Cologne, Germany), monensin was from Calbiochem (Frankfurt am Main/Germany). All other chemicals used in this study were obtained in the highest quality available from Sigma (Munich, Germany).

Synaptosomal Uptake Experiments

Female NMRI mice were sacrificed by decapitation and the brains immediately dissected on ice. Total brains were prepared for ^{3}H-GABA- or forebrains for ^{3}H-L-glutamate uptake experiments. The tissue was homogenized in 15 ml ice-cold sucrose solution (0.32 M) in a Potter-Elvjehem glass homogenizer plus teflon pestle (Braun Melsungen, Germany) by ten strokes at 300 rpm and diluted with further 10 ml of the sucrose medium. The nuclear fraction was eliminated by centrifugation (10 minutes at 750 x g; 0-4°C) and the supernatant was centrifuged (20 minutes at 17400 x g; 0-4°C) to obtain the crude synaptosomal pellet. The pellet was resuspended in ice-cold 4-(2-hydroxyethyl)-1-piperazineethansulfonic acid (HEPES) buffer (NaCl: 150; HEPES: 10; KCl: 6.2; Na_2HPO_4: 1.2; glucose: 10 mM; pH 7.4 at 37°C) to a final concentration of 28 mg/ml or 15 mg/ml wet weight for ^{3}H-GABA- or for ^{3}H-L-glutamate uptake. Protein content was determined according to the method of Bradford (1976). Aliquots of the suspension were added into 96-well microtiter plates containing ice-cold HEPES buffer together with varying concentrations of drugs affecting uptake. The plates were preincubated at 37°C for 15 minutes in a slightly shaking water bath, then cooled in ice water for 5 minutes. Uptake was initiated by addition of the ^{3}H-labeled ligands (^{3}H-GABA: 0.5, ^{3}H-L-glutamate: 1 nM) to a final volume of 500 µl per well. After incubation for 4 minutes at 37°C, for which period specific uptake was linearly correlated with incubation time, cooling on ice for 5 minutes terminated the uptake. The samples were filtered under slight vacuum (Whatman GF/B glass fiber filters) and washed three times for one second (4°C) using a 24-sample Brandel Cell Harvester (^{3}H-GABA: HEPES buffer; ^{3}H-L-glutamate: saline 0.9%). Non-specific uptake was estimated in parallel probes containing unlabeled neurotransmitters (1mM) (Enna and Snyder 1975; Robinson et al. 1991). Non-specific uptake was similar when determined by incubation at 4°C. In most cases data were normalized as "percent of specific uptake", always referring to the specific uptake obtained from total minus non-specific uptake. Filters were removed and placed in plastic vials containing 4 ml Lumasafe plus (Packard, Dreieich, Germany) and radioactivity was determined in a TR 1900 scintillation counter (Canberra-Packard, Dreieich, Germany).

Table 1. IC_{50}-Values and Hill Coefficients of Reference Substances for the Specific Synaptosomal Uptake of ^{3}H-L-Glutamate and ^{3}H-GABA. Data are Means ± SD of 3–5 Independent Experiments

^{3}H-L-glutamate Uptake			^{3}H-GABA Uptake		
Inhibitor	**IC_{50} [μM]**	**n_H**	**Inhibitor**	**IC_{50} [μM]**	**n_H**
L-glutamate	4.28 ± 0.15	−1.148 ± 0.295	GABA	9.75 ± 1.89	−0.853 ± 0.112
DL-threo-β-hydroxy-aspartate	1.33 ± 0.31	−0.919 ± 0.237	DABA	26.67 ± 18.94	−0.807 ± 0.161
Pyrrolidone-2, 4-dicarbonic acid	1.53 ± 0.23	−1.079 ± 0.265	β-alanine	>1000	
Dihydro kainic acid	>200				
α-aminoadipate	>100				
D-glutamate	>100				
NMDA	>100				

Kinetic analyses were performed accordingly by addition of 0.4–25 nM ^{3}H-GABA or 2.6–85 nM ^{3}H-L-glutamate.

Data Analyses

Data was calculated using iterative curve fitting routines (Graph Pad® Prism ver. 2.01/1996). Individual group differences were assessed as appropriate using paired two-tailed Student's *t*-test. Differences were deemed significant when $p < .05$.

RESULTS

The strong inhibition of ^{3}H-L-glutamate uptake by pyrrolidone (2,4) dicarboxylic acid and of ^{3}H-GABA uptake by diaminobutyric acid (DABA) and the weak inhibition of ^{3}H-GABA uptake by β-alanine (Table 1) and of ^{3}H-L-glutamate by several other compounds suggests that synaptosomal uptake of both neurotransmitters is mainly associated with neuronal uptake sites (Enna and Snyder 1975; Lester and Peck 1979; Nakashita et al. 1997; Sutton and Simmonds 1974; Robinson et al. 1991; Rauen et al. 1992). In agreement with our previous findings (Chatterjee et al. 1998a), hyperforin inhibits both uptake systems with IC_{50}-values in the nanomolar range (Figures 1 and 2, Table 2) None of several typical antidepressants investigated showed a relevant effect on both uptake systems (Table 3), especially considering their much lower IC_{50}-values for the serotonin and/or norepinephrine transporters (Tatsumi et al. 1997; Richelson and Pfenning 1984).

The inhibition of the uptake of both amino acids by

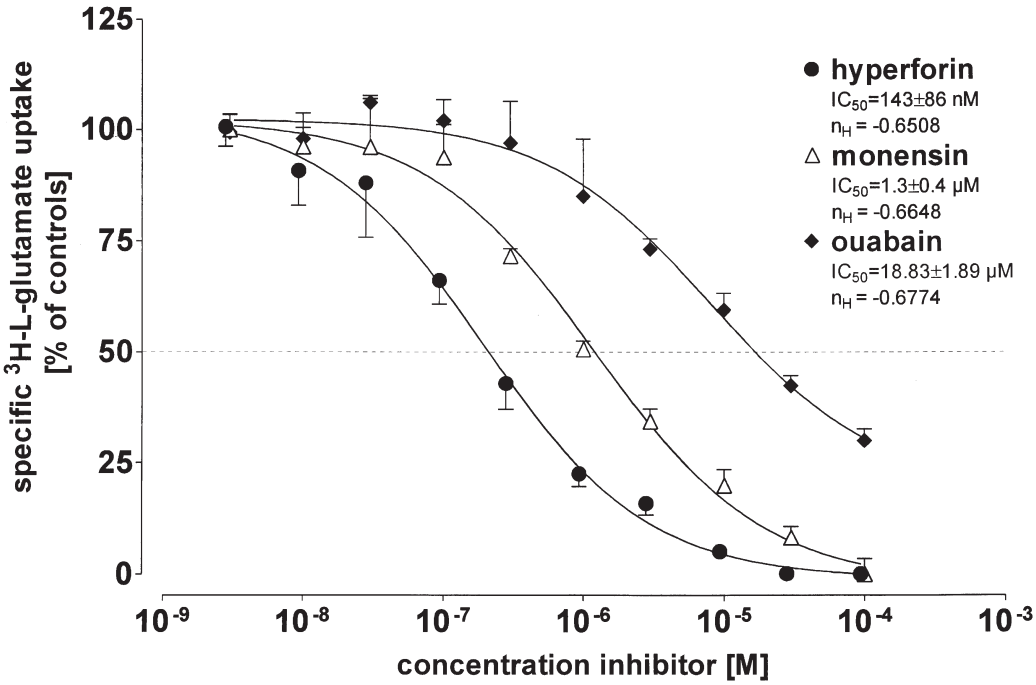

Figure 1. Inhibition of ^{3}H-L-glutamate uptake by hyperforin, monensin and ouabain. Data are means ± SD of 3–6 independent experiments each done in triplicate and are given as percent of specific uptake (see methods).

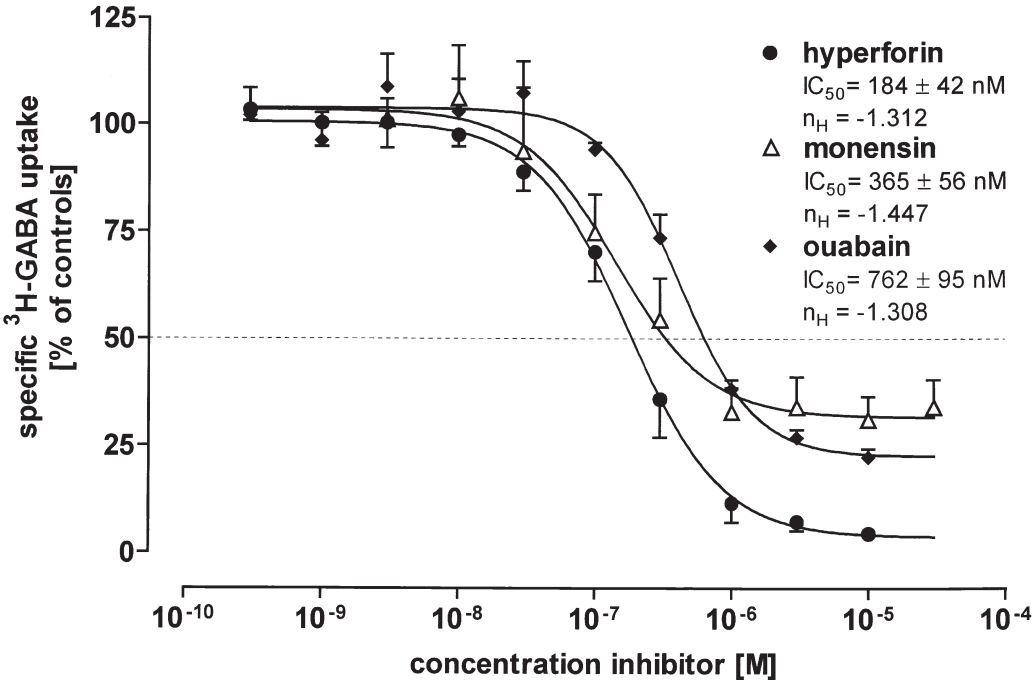

Figure 2. Inhibition of ^{3}H-GABA uptake by hyperforin, monensin and ouabain. Data are means ± SD of 3–6 independent experiments each done in triplicate and are given as percent of specific uptake (see methods).

hyperforin was at least partially reversible, since washing the synaptosomes only once (Table 4) could significantly reduce inhibition. The inhibition of both uptake systems by hyperforin was associated with a significant decrease of V_{max}, while K_m-values were not significantly altered (Figure 3, Table 5), suggesting non-competitive inhibition.

We have previously shown that the inhibition of serotonin uptake in human platelets is associated with an elevation of the free intracellular sodium concentration and can be mimicked by the non-specific sodium ionophore monensin (Singer et al. 1999). Monensin also inhibited the synaptosomal uptake of ^{3}H-L-glutamate (Figure 1) and ^{3}H-GABA (Figure 2) with Hill coefficients similar to those of hyperforin. As hyperforin is not a sodium ionophore by itself (Singer et al. 1999) we additionally investigated, if its uptake inhibition is associated with mechanisms regulating the physiological sodium conductance.

However, blocking voltage dependent sodium channels (tetrodotoxin (TTX) up to a concentration of 1 μM) had no effect on both uptake systems and also did not modify the effect of hyperforin (data not shown). Ouabain and digoxin inhibited both uptake systems as hyperforin does (Table 2). An alternative mechanism could be the participation of amiloride sensitive sodium conductive pathways (Na^+ channels and Na^+-H^+-antiporters). Accordingly, the amiloride analogues at low concentrations significantly enhanced ^{3}H-L-glutamate

Table 2. Inhibition of Specific ^{3}H-L-Glutamate and Specific ^{3}H-GABA Uptake by Hyperforin and Several Drugs Affecting Sodium Conductance. Data are Means ± SD of 4–8 Independent Experiments

	^{3}H-GABA Uptake	^{3}H-L-glutamate Uptake
	IC$_{50}$[μM]	IC$_{50}$[μM]
hyperforin	0.184 ± 0.042	0.143 ± 0.086
monensin	0.356 ± 0.056	1.30 ± 0.36
ouabain	0.762 ± 0.095	18.83 ± 1.89
digoxin	6.21 ± 3.03	89.25 ± 36.68
TTX	n.e.[a]	n.e.[a]
amiloride	>100	>100
MIA	20.30 ± 4.79	27.77 ± 5.03
EIPA	25.59 ± 2.88	20.41 ± 4.38
benzamil	90.28 ± 44.48	>100

[a]no effect at 100 μM

Table 3. Inhibition of Specific ^{3}H-L-Glutamate and Specific ^{3}H-GABA Uptake by Several Typical Antidepressant Drugs. Data are Means ± SD of 3–5 Independent Experiments

	^{3}H-GABA Uptake	^{3}H-L-glutamate Uptake
	IC$_{50}$[μM]	IC$_{50}$[μM]
amitriptyline	4.56 ± 0.17	39.89 ± 0.14
citalopram	>100	>100
clomipramine	>100	33.21 ± 0.11
desipramine	>100	>100
imipramine	>100	>100

Table 4. Reversibility of ^{3}H-GABA- and ^{3}H-L-Glutamate Uptake Inhibition by Hyperforin, Monensin and a Competitive Inhibitor of each Transporter (for Further Details see Text). Since Washing Alone Reduces the Synaptosomal Levels of Both Amino Acids (about 50%), Data are Normalized as Percent of the Respective Controls (Uptake before and after Washing). Data are Means ± SD of 12–17 Independent Experiments

^{3}H-GABA uptake		Specific uptake [% of respective controls]	
inhibitor	**Conc [μM]**	**unwashed**	**washed 1 ×**
hyperforin	1	11.32 ± 4.14	35.55 ± 5.40***
monensin	0.3	59.98 ± 14.54	85.35 ± 17.35***
DABA	250	25.74 ± 7.12	71.11 ± 36.92***
^{3}H-L-glutamate uptake		**Specific uptake [% of respective controls]**	
inhibitor	**Conc [μM]**	**unwashed**	**washed 1 ×**
hyperforin	1	20.38 ± 12.12	77.06 ± 20.52***
monensin	0.75	61.88 ± 14.97	97.81 ± 13.42***
D-glutamate	150	39.65 ± 14.37	88.26 ± 19.30***

***$p < .001$.

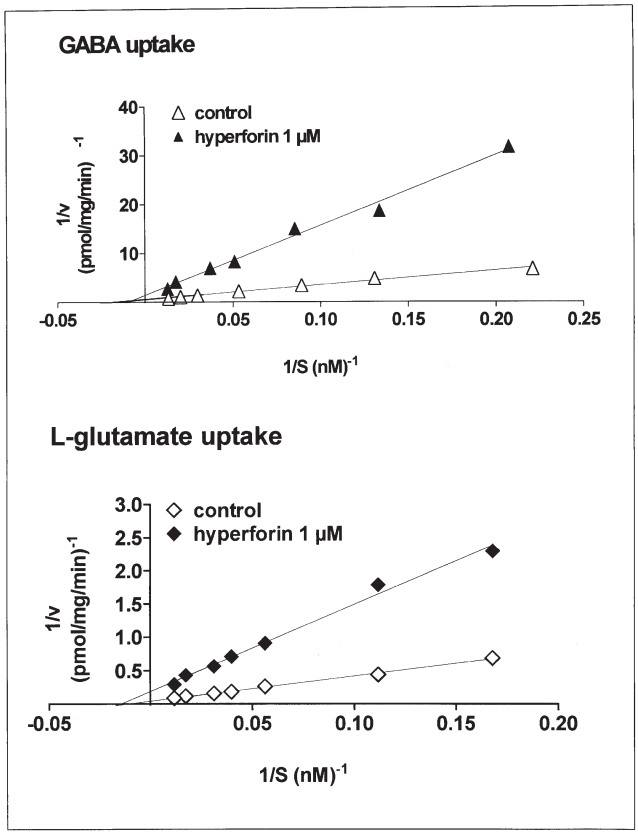

Figure 3. Lineweaver-Burk plots of the inhibition of specific ^{3}H-GABA and ^{3}H-L-glutamate uptake inhibition by hyperforin. The graphs show single experiments representative for n = 5–6 (GABA) or n = 9–10 (L- glutamate) independent determinations ($R^2 > 0.99$) all done in triplicate. Data are corrected for the protein content. ^{3}H-GABA uptake: △ V_{max} = 1.36 pmol/mg/min, K_m = 60.83 nM; ▲ V_{max} = 0.54 pmol/mg/min, K_m = 60.32 nM ; ^{3}H-L-glutamate uptake: ◇ V_{max} = 6.13 pmol/mg/min, K_m = 85.67 nM ; ◆ V_{max} = 1.55 pmol/mg/min; K_m = 81.75 nM.

uptake (Figures 4, 6, and 7) and also inhibited uptake at high concentrations not related to the inhibition of Na^+ channels and/or Na^+-H^+ -antiporters (Frelin et al. 1988). (Figure 4, Table 2). Even more important, the same low concentration of these compounds which itself enhanced ^{3}H-L-glutamate uptake, significantly attenuated the uptake inhibition by hyperforin, but not by monensin and ouabain (Figure 5). A comparable observation was made for the ^{3}H-GABA uptake, although the effects were less clearly pronounced (Table 5). Moreover, a typical shift to the right of the dose response curves of hyperforin (concentration range >1μM) was observed by EIPA or benzamil, two amiloride derivatives with a more than ten times higher affinity for either Na^+-H^+-antiporters or for sodium channels, respectively (Figures 6 and 7) (Frelin et al. 1988).

DISCUSSION

As indicated by the IC_{50}-values of well characterized competitors (Table 1), the synaptosomal uptake systems used in the present communication seem to be mainly associated with the neuronal GABA transporter GAT1 (high affinity of DABA, low affinity of β-alanine) (Nakashita et al. 1997; Enna and Snyder 1975; Lester and Peck 1979) or with the neuronal L-glutamate transporter EAAC 1 (high affinity of pyrrolidone 2,4-dicarboxylic acid) (Robinson et al. 1991; Rauen et al. 1992). As there is only some homology between both classes of transporter molecules (Malandro and Kilberg 1996) there is no overlap between the specific inhibitors of GABA or L-glutamate transporters, respectively (see also Table 1).

By contrast, hyperforin inhibits both synaptosomal uptake systems with rather similar IC_{50}-values in the

Table 5. Kinetic analyses of the inhibition of the specific synaptosomal uptake of ^{3}H-L-glutamate and ^{3}H-GABA by hyperforin. Data for V_{max} and K_m are means ± SD of (N) independent experiments

	Conc. [μM]	V_{max} [pmol/mg/min]	K_m [nM]	N
^{3}H-GABA	0	2.76 ± 1.91	100.48 ± 88.64	9
	0.5	0.87 ± 0.62*	85.15 ± 89.69	9
	1	0.77 ± 0.41*	86.72 ± 60.99	9
^{3}H-L-glutamate	0	8.27 ± 2.66	143.13 ± 62.03	8
	0.2	4.76 ± 2.30*	154.25 ± 109.69	8
	1	1.80 ± 0.92***	84.25 ± 40.79	8

*$p < .05$.
***$p < .001$.

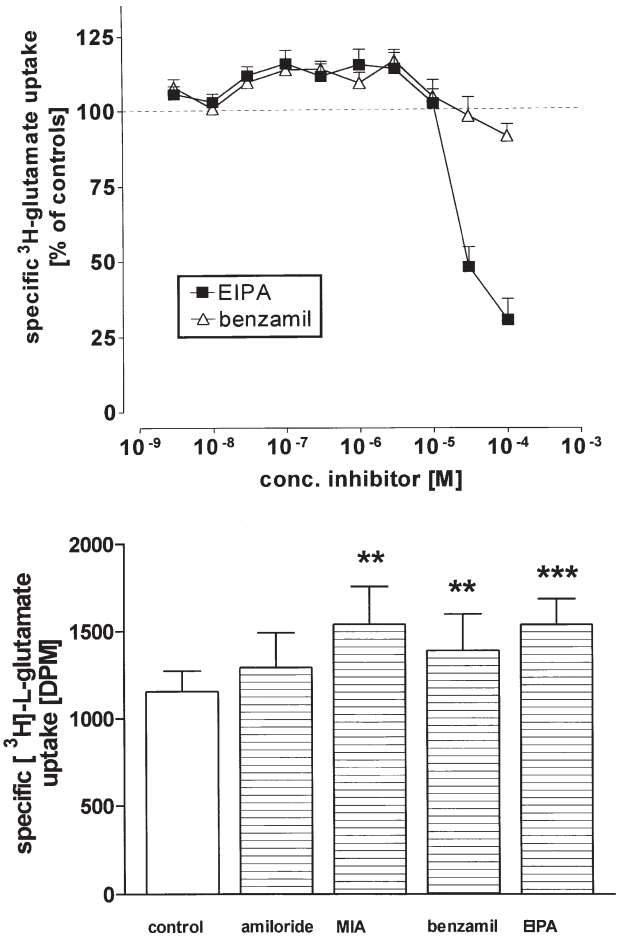

Figure 4. (*top*) Effect of increasing concentrations of two amiloride derivatives on specific ^{3}H-L-glutamate uptake (n = 3–6). (*bottom*) Maximum stimulation of specific ^{3}H-L-glutamate uptake by several amiloride derivatives. Data are means ± SD of 11–16 independent determinations and were done in triplicate and are given as percent of specific uptake (see methods).

high nanomolar range, close to its IC_{50}-values for serotonin, norepinephrine and dopamine uptake (Chatterjee et al. 1998a). This contrasts to all clinically used antidepressant drugs, which are only weak inhibitors of L-glutamate and GABA uptake, especially in relationship to their potent inhibition of serotonin and/or norepinephrine uptake (Tatsumi et al. 1997; Richelson and Pfenning 1984). Some experimental findings indicated that administration of hypericum extract or of hyperforin lead to an enhanced glutamatergic and GABAergic neurotransmission in animal or human brain (Dimpfel et al. 1998; Schellenberg et al. 1998; Kaehler et al. 1999). However, the possible relevance of these effects for the antidepressant activity is not yet known.

Uptake inhibition of ^{3}H-L-glutamate and ^{3}H-GABA by hyperforin is clearly non-competitive excluding simple substrate competition for the ligand binding sites, but is at least partially reversible by a single washing

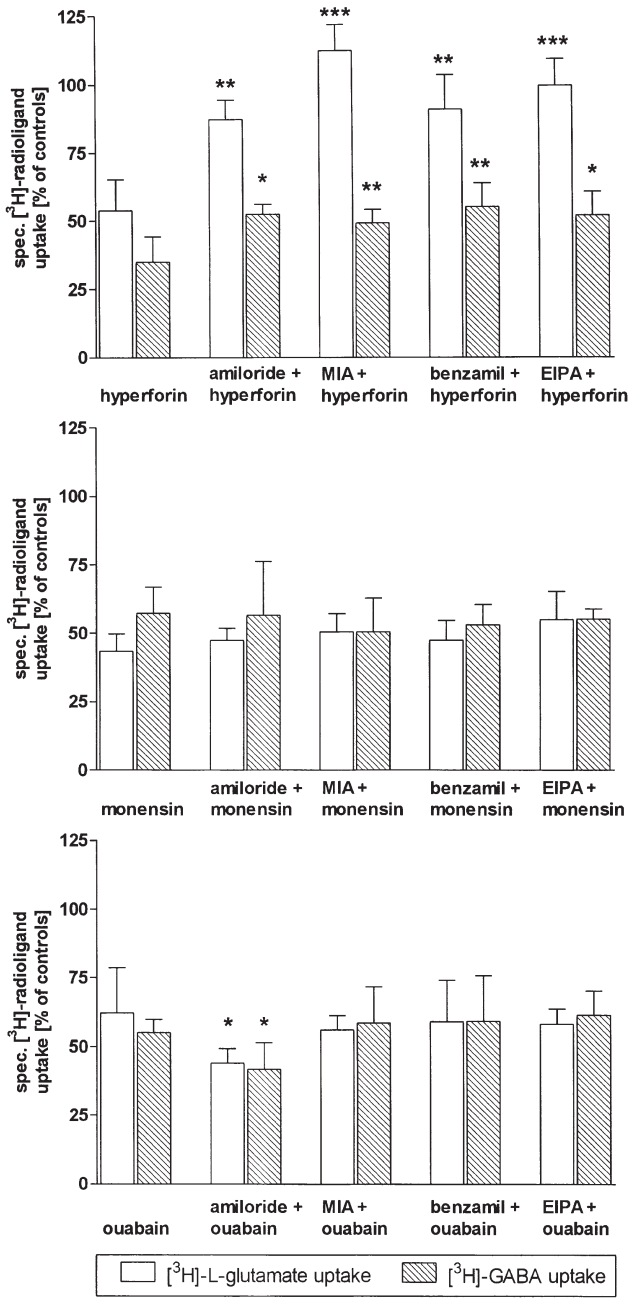

Figure 5. Effects of several amiloride analogues on the inhibition by hyperforin (top), monensin (middle) or ouabain (bottom) of ^{3}H-L-glutamate and ^{3}H-GABA uptake. Data are means ± SD of 6–9 independent determinations and were done in triplicate. Data are always given as percent of specific uptake without the presence of any inhibitor. Concentrations of hyperforin, monensin and ouabain were always 0.25μM, 0.35μM and 10μM, respectively. The concentrations of the analogues are amiloride 10μM, MIA 10μM, benzamil 3μM and EIPA 3μM for the L-glutamate uptake and amiloride 10μM, MIA 1μM, benzamil 3 μM and EIPA 10μM for the GABA uptake.

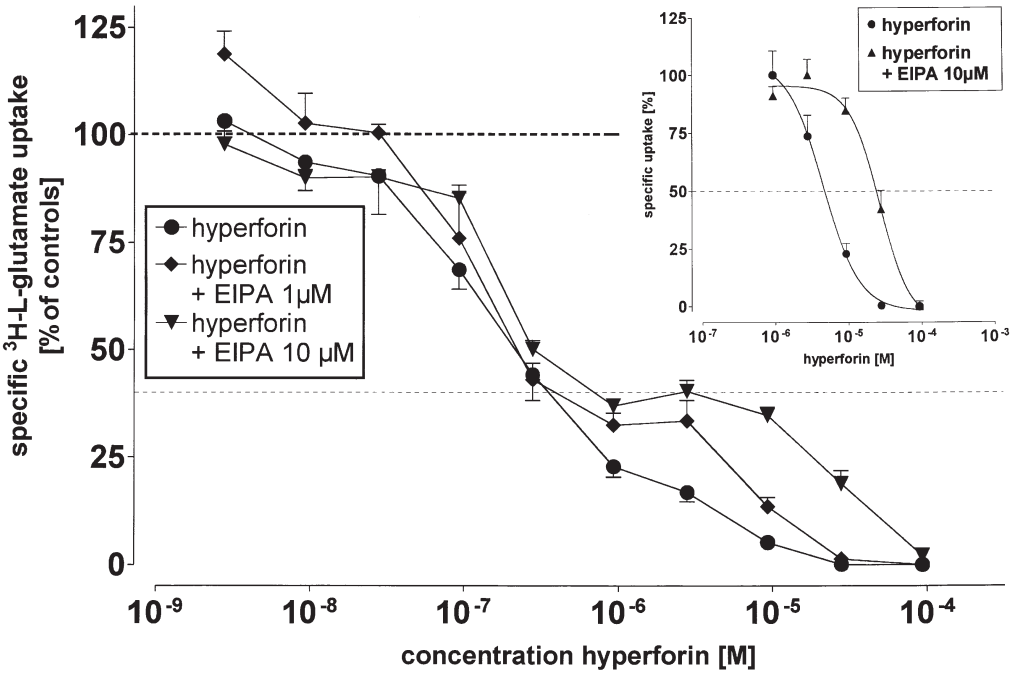

Figure 6. Effects of EIPA (1μM, 10μM) on the dose response curve of hyperforin as an inhibitor of specific ^{3}H-L-glutamate uptake. Data are means ± SD of six independent determinations each done in triplicate and are given as percent of specific uptake (see methods). Inset: Uptake inhibition by hyperforin alone or in the presence of EIPA (10μM). Data are normalized for the inhibition by hyperforin at 1μM (=100%). ● hyperforin: IC_{50} = 4.54 ± 1.24 μM; ▲ hyperforin+ EIPA 10 μM:IC_{50} = 26.61 ± 11.79 μM.

experiment, which also excludes unspecific damage of the synaptosomes as major mechanism. Taking together these observations suggest that the mechanism of action of hyperforin is probably not associated with specific binding to the different transporter molecules, but with mechanisms relevant for the activity of neurotransmitter transporters in general. The latter assumption could explain that hyperforin inhibits the synaptosomal uptake not only of ^{3}H-GABA and ^{3}H-L-glutamate, but also the uptake of serotonin, norepinephrine and dopamine with IC_{50}-values in the nanomolar range (Müller et al. 1998; Chatterjee et al. 1998a).

The sole driving force of all neurotransmitter transporters is the Na^+ -gradient, as the transporters are operated by Na^+-cotransport (Malandro et al. 1996). Conditions which decrease the Na^+-gradient of the neuronal membrane, either by lowering extracellular or by elevating intracellular Na^+, are well known to inhibit the neuronal neurotransmitter transporters. While extracellular Na^+ was kept constant in our experiments, we investigated if hyperforin works via mechanisms regulating sodium conductance. The Na^+-gradient is primarily maintained by Na^+-K^+-ATPase. It is well known that by blocking this enzyme with high concentrations of ouabain or digoxin, synaptosomal uptake can be inhibited and that under non-depolarizing conditions voltage-dependent Na^+ channels are not involved. Our experiments with ouabain or digoxin and with TTX are simply confirming these facts. However, preliminary experiments indicate that hyperforin is not an inhibitor of Na^+-K^+- ATPase (Chatterjee, personal communication, Eckert and Müller, unpublished findings). Moreover, neither ouabain nor TTX at rather high concentrations did modify the inhibition of ^{3}H-L-glutamate and ^{3}H-GABA uptake by hyperforin. Taken together, it seems very unlikely that hyperforin works via Na^+-K^+-ATPase inhibition or activation of voltage-dependent sodium channels.

Synaptosomal uptake of ^{3}H-GABA and ^{3}H-L-glutamate can be inhibited by the Na^+ ionophore monensin as it has already been reported for serotonin and dopamine (Itzenwasser et al. 1982; Reith and O'Reilly 1990; Singer et al. 1999). However, recent findings using human platelets indicate that hyperforin is not a non-specific physiochemical Na^+-ionophore, since it elevates the intracellular Na^+ concentration to a certain level only, contrasting monensin which leads to complete equilibrium between the intracellular and the extracellular Na^+ levels (Singer et al. 1999).

An important system regulating intracellular Na^+ concentrations is the Na^+-H^+-exchanger, which can be specifically inhibited by amiloride and several of its analogues (Grinstein and Rothstein 1986; Frelin et al. 1988). Na^+-H^+-exchangers have also been identified on brain cell membranes and synaptosomes (Kalaria et al. 1998; Sauvaigo et al. 1984; Sanchez-Armass et al. 1994). Inhibition by amiloride or amiloride derivatives re-

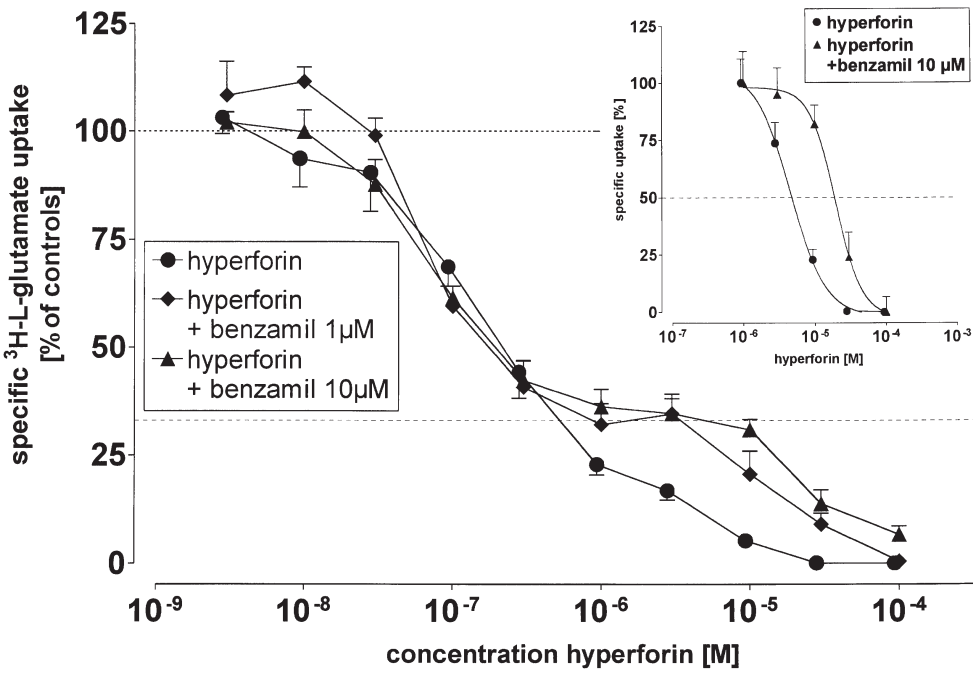

Figure 7. Effects of benzamil (1µM, 10µM) on the dose response curve of hyperforin as an inhibitor of specific L-glutamate uptake. Data are means ± SD of six independent determinations each done in triplicate (see Figure 6). Inset: (see Figure 6) ● hyperforin: IC_{50} = 4.54 ± 1.24 µM; ▲ hyperforin+ benzamil 10 µM: IC_{50} = 19.5 ± 11.48 µM.

duces synaptosomal Na^+ uptake leading to a reduced free intracellular Na^+ concentration (Sauvaigo et al. 1984; Liu et al. 1996). In agreement with latter findings, incubation of synaptosomes with amiloride derivatives significantly enhanced ^{3}H-L-glutamate uptake at concentrations relevant for inhibiting the Na^+-H^+-exchanger (1–10 µmol/L) (Frelin et al. 1988). At much higher concentrations, these compounds showed nonspecific inhibition of ^{3}H-L-glutamate uptake. Very interestingly, when synaptosomes were incubated with all four drugs in the presence of a hyperforin concentration giving about 50% inhibition, the effect of hyperforin was significantly attenuated in all cases and was even abolished in the case of methylisobutylamiloride (MIA). Even more important, none of the compounds changed the uptake inhibition by monensin and ouabain, except a small additive enhancement of the ouabain inhibition by amiloride. In principle, the same findings were made with all four compounds for ^{3}H- GABA uptake. These findings would be compatible with the assumption that hyperforin activates the Na^+-H^+-exchanger and that this effect is inhibited by amiloride or its derivatives. Initial findings with human platelets (Singer and Müller, unpublished observations), where hyperforin elevates intracellular pH after 15 minutes incubation and findings of S.S. Chatterjee with smooth muscle cells in tissue culture (personal communication), where hyperforin leads to an acidification of the medium, are in line with these assumptions.

Unfortunately, amiloride and its derivatives are not specific for the Na^+-H^+-exchanger, but are also antagonists of amiloride sensitive "epithelial" Na^+ channels (Frelin et al. 1988). At least one subclass of these channels (brain sodium channels, BNC1 and BNC2) are expressed in the brain (Garcia-Anoveros et al. 1997; Price et al. 1996). In order to investigate the possible relevance of these ion channels, additional experiments were made with two of the derivatives, EIPA and benzamil. 1 µM EIPA showed maximum inhibition of the Na^+-H^+-exchanger in two different tissues (Frelin et al. 1988; Sauvaigo et al. 1984) and displaces specific ^{3}H-MIA binding to the exchanger with a K_i of 0.14 µM (Kalaria et al. 1998). Much higher concentrations are needed to inhibit sodium channels (Frelin et al. 1988). Its effect on the dose response curve of hyperforin as an inhibitor of synaptosomal ^{3}H-L-glutamate uptake is biphasic (Figure 7). At 1 µM EIPA, where the exchanger is blocked but not the sodium channels, EIPA stimulates uptake. This effect is antagonized already by very low hyperforin concentrations. The inhibitory effect of hyperforin on ^{3}H-L-glutamate uptake at higher hyperforin concentrations (1–100 µM) is only slightly attenuated by EIPA (1 µM), but is profoundly reduced by an EIPA concentration of 10 µM. Conversely, benzamil blocks sodium channels already at a concentration of 1 µM (Frelin et al. 1988). Much higher concentrations are needed to inhibit the Na^+-H^+-exchanger (Frelin et al. 1988). Its IC_{50} for inhibiting specific ^{3}H-MIA binding is about 25 µM (Kalaria et al. 1998). Similar to EIPA, 1 µM

benzamil is sufficient for maximal stimulation of ^{3}H-L-glutamate uptake in the absence of hyperforin, but contrary to EIPA, 1 μM benzamil is nearly sufficient for attenuating the effect of hyperforin (1–100 μM) on ^{3}H-L-glutamate uptake. Increasing the benzamil concentration up to 10 μM has little further effect. These findings would suggest that the increase of ^{3}H-L-glutamate uptake, which is seen without hyperforin or at very low hyperforin concentrations, could be elicited by inhibition of either the Na^+-H^+-exchanger or of amiloride sensitive sodium channels . However, the pronounced shift to the right of the dose response curves of hyperforin (insets in Figures 6 and 7) might be mainly due to a blockade of sodium channels.

Whether hyperforin activates both systems directly or indirectly, e.g. by altering H^+ concentration, as both systems are pH dependent, is currently under investigation (see also the discussion above). In conclusion, hyperforin clearly shows a typical preclinical antidepressant profile (see introduction), but seems to work at the cellular level by a completely novel mechanism related to sodium conductive pathways. If this mechanism contributes directly to the preclinical antidepressant profile beyond the rather unselective reuptake inhibition is possible, but needs further experimental work.

ACKNOWLEDGMENTS

This study was supported by research grants of Lichtwer AG (Berlin, Germany), Dr. Willmar Schwabe GmbH & Co (Karlsruhe, Germany) and by the Fonds der Chemischen Industrie.

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