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The antidepressant hyperforin increases the phosphorylation of CREB and the expression of TrkB in a tissue-specific manner

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Abstract

Hyperforin is one of the main bioactive compounds that underlie the antidepressant actions of the medicinal plant *Hypericum perforatum* (St. John's wort). However, the effects of a chronic hyperforin treatment on brain cells remains to be fully addressed. The following study was undertaken to further advance our understanding of the biological effects of this plant extract on neurons. Special attention was given to its impact on the brain-derived neurotrophic factor (BDNF) receptor TrkB and on adult hippocampal neurogenesis since they appear central to the mechanisms of action of antidepressants. The consequences of a chronic hyperforin treatment were investigated on cortical neurons in culture and on the brain of adult mice treated for 4 wk with a daily injection (i.p.) of hyperforin (4 mg/kg). Its effects on the expression of the cyclic adenosine monophosphate response element-binding protein (CREB), phospho-CREB (p-CREB), TrkB and phospho-TrkB (p-TrkB) were analysed by Western blot experiments and its impact on adult hippocampal neurogenesis was also investigated. Hyperforin stimulated the expression of TRPC6 channels and TrkB via SKF-96365-sensitive channels controlling a downstream signalling cascade involving Ca^{2+} , protein kinase A, CREB and p-CREB. *In vivo*, hyperforin augmented the expression of TrkB in the cortex but not in the hippocampus where hippocampal neurogenesis remained unchanged. In conclusion, this plant extract acts on the cortical BDNF/TrkB pathway leaving adult hippocampal neurogenesis unaffected. This study provides new insights on the neuronal responses controlled by hyperforin. We propose that the cortex is an important brain structure targeted by hyperforin.

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Key words: Antidepressants, CREB, neurogenesis, PKA, TrkB, TRPC6.

Introduction

The medicinal plant *Hypericum perforatum*, also named St. John's wort (SJW), contains nearly two dozen of bioactive compounds which possess interesting pharmacological properties including: anti-bacterial,

anti-inflammatory, anti-tumoural and anti-angiogenic properties (Lorusso *et al.* 2009; Saddiqe *et al.* 2010; Schempp *et al.* 1999). In addition, SJW extracts have been shown to possess central nervous system (CNS) effects and are used as antidepressants (Di Carlo *et al.* 2001; Linde *et al.* 1996; Mennini & Gobbi, 2004; Muller, 2003). Although several constituents of SJW seem to contribute to its therapeutic efficacy (Chatterjee *et al.* 1998b), the phloroglucinol derivative hyperforin is regarded as the main effector (Cervo *et al.* 2002; Chatterjee *et al.* 1998a; Zanolini, 2004). However, its actions on brain cells and the mechanisms

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underlying its putative clinical antidepressant effect remain poorly characterized. Recently, many cellular targets of hyperforin have been identified (Beerhues, 2006; Medina *et al.* 2006). For instance, it has been determined that this plant extract controls the activity of enzymes like microsomal prostaglandin E2 synthase-1 (Koeberle *et al.* 2011), sirtuins 1 and 2 (Gey *et al.* 2007), cyclooxygenase-1 or 5-lipoxygenase (Albert *et al.* 2002), as well as binding to intracellular receptors like the pregnane X receptor (Moore *et al.* 2000) and altering the expression of genes, e.g. *CYP2C* genes (Chen & Goldstein, 2009). Hyperforin has also been shown to interfere with neurotransmission by inhibiting the re-uptake of many neurotransmitters and dissipating the pH gradient of synaptic vesicles. This results in a redistribution of monoamines from these vesicles to the cytoplasmic compartment (Roz & Rehavi, 2004). Another interesting characteristic of hyperforin is its ability to influence the transport of ions. Hyperforin is a potent blocker of various channels like ionotropic GABA and glutamate receptors (Chatterjee *et al.* 1999; Fisunov *et al.* 2000; Kumar *et al.* 2006). Besides these inhibitory properties, hyperforin is currently known to activate TRPC6, a Ca^{2+} -conducting channel of the plasma membrane (Leuner *et al.* 2007). TRPC6 is the only channel opened by this plant extract. The hyperforin-dependent activation of TRPC6 channels controls various biological processes. For instance, when chronically applied, hyperforin promotes neurite extension in the neuronal cell line PC12 (Leuner *et al.* 2010) and induces the differentiation of keratinocytes (Muller *et al.* 2008).

The aim of the present study was to advance our understanding of hyperforin's mechanisms of action on brain cells. In particular, special attention was given to its impact on the brain-derived neurotrophic factor (BDNF) receptor TrkB and on adult hippocampal neurogenesis since they appear central in the molecular pathophysiology of depression and in the pharmacological mechanisms of action of antidepressants (Duman & Monteggia, 2006; Kozisek *et al.* 2008; Miguel-Hidalgo & Rajkowska, 2002; Sahay & Hen, 2007). Thus, the question of whether hyperforin affects the above-mentioned processes was addressed by using neurons in culture and adult mice chronically treated with hyperforin. The data collected illustrate that chronic hyperforin treatment triggers an intracellular signalling pathway that involves the cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA) and the transcription factor cAMP response element-binding protein (CREB), and regulates the expression of the BDNF receptor TrkB and TRPC6. This hyperforin-dependent cascade critically

depends on the activity of Ca^{2+} -conducting channels and occurs specifically in the cortex but not in the hippocampus. Our findings provide new insights into the neurobiological actions of a chronic hyperforin treatment on CNS neurons.

Methods

Cell cultures

Primary cultures of cortical neurons were prepared from embryonic E13 C57BL6/J mice (Bouron *et al.* 2005). Brains were placed in an ice-cold Ca^{2+} - and Mg^{2+} -free Hank's solution to which was added 33 mM glucose, 4.2 mM NaHCO_3 , 10 mM Hepes, and 1% penicillin/streptomycin. Depending on the number of embryos, 1–3 pregnant mice were used to prepare one batch of cultured cells. The cortices were pooled and placed in a sterile 1.5 ml Eppendorf tube before being dissociated by means of repetitive aspirations through a sterile and fire-polished Pasteur pipette. This cell suspension was then filtered through a 40 μm cell strainer (BD Falcon, USA) and plated onto poly-L-ornithine-treated Petri dishes (\varnothing 6 cm). Cells were grown for 3 d in a Neurobasal medium containing 2% B27, 1% penicillin/streptomycin and 500 μM glutamine (Bouron *et al.* 2005). Next, half of this culture medium was removed and replaced with fresh Neurobasal medium. The procedures used were approved by the Ethical Committee of Grenoble (ComEth) (protocol 7_IRTSV-LCBM-AB-01).

Chronic treatment of C56Bl6/J mice with hyperforin

Twenty-six 5-month-old male C57BL6/J mice (Charles River, France) were housed under standard conditions on a 12-h light/dark cycle (lights on 07:00 hours) with water and food available *ad libitum*. Thirteen animals received a daily intraperitoneal (i.p.) injection of a fresh hyperforin solution (4 mg of hyperforin/kg body weight) diluted in a sterile NaCl (0.9%) solution. Control animals ($n=13$) were treated with a hyperforin-free NaCl solution. After 4 wk of treatment, the brain of three hyperforin-treated mice and of three control mice were isolated and placed in an ice-cold saline solution. They were subsequently used for the extraction of proteins (see below). The other animals received two 5-bromo-2'-deoxyuridine (BrdU) injections at 24-h intervals (25 mg BrdU/animal). After this, the daily injections of hyperforin (or NaCl) were repeated for 3 more weeks before intra-cardiac injection of paraformaldehyde (PFA) and processing of the brains for analysis of hippocampal neurogenesis (see below).

Protein extraction and Western blot analysis

For the *in-vivo* experiments, proteins from the cortex and the hippocampus were extracted using Nucleo-spin RNA/protein kit (Macherey-Nagel, France) followed by precipitation with ethanol. Samples were kept on ice for 30 min and centrifuged at 11 320 g for 30 min at 4 °C. The pellet was resuspended in a buffer containing 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.24% Tris-HCl and 0.5% deoxycholate.

For the *in-vitro* experiments, hyperforin was added to the culture medium 5 d after the plating of the neuronal cells. At that time, the culture medium was removed and replaced by a fresh Neurobasal medium containing 1 μ M hyperforin (or DMSO, control cells). The concentration of DMSO was <0.1%. Cells were subsequently kept for 72 h during which the culture medium containing hyperforin (or DMSO) was changed every day. At the end of the 72-h treatment, cells were harvested and centrifuged for 5 min at 453 g. The pellet was resuspended in a buffer containing (in mM): 10 Hepes, 3 MgCl₂, 40 KCl, 2% glycerol, 1% Triton X-100, 1% protease inhibitors (Sigma, France), with a pH of 7.5. The samples were kept on ice for 30 min and centrifuged at 11 320 g for 30 min at 4 °C before collecting the supernatant. Proteins were quantified using the Bio-Rad DC protein assay.

Immunoblotting analyses were performed with 50 μ g proteins after separation on a 8% SDS-polyacrylamide gel. PVDF membranes were blocked for 2 h in 5% non-fat dry milk. The primary antibodies used were either anti-TrkB (1:500, Millipore, USA), anti-phospho-TrkB (anti-p-TrkB) (1:200, tebu-bio, France), anti-CREB (1:1000, Millipore), anti-phospho-CREB (anti-p-CREB) (1:1000, Millipore), anti-TRPC6 (1:200, Alomone Labs) or actin (1:250, Sigma). Overnight incubation at 4 °C with the primary antibody was followed by incubation with a horseradish peroxidase-coupled secondary antibody (1:5000, Bethyl, USA). Detection of the bands by chemiluminescence (ECL, Pierce, USA) was achieved using a Fusion Fx7 apparatus (Vilbert Lourmat, France). Quantification of protein expression was made with ImageJ software: for a given protein, its expression was normalized to the level of actin expression.

Adult hippocampal neurogenesis

For these experiments, 10 animals from each group (i.e. hyperforin-treated and NaCl-treated animals) were used. Animals were anaesthetized with pentobarbital. The fixation of the brain was achieved after an intra-cardiac injection of 4% PFA in PBS (Dupret *et al.* 2008). Free-floating sections (40 μ m) were

processed in a standard immunohistochemical procedure in order to visualize BrdU (1:2000, Accurate Scientific, USA), phosphorylated histone H3 (pH 3, 1:2000, Upstate, USA) and doublecortin (DCX, 1:1000, Santa Cruz Biotechnology, USA), on alternate one-in-ten sections. The numbers of immunoreactive (IR) cells throughout the entire granular layer of the supragranular and infragranular blades of the dentate gyrus (both sides for BrdU and HH3, and the left side for DCX) were estimated using the optical fractionator method (Dupret *et al.* 2007).

Statistical analysis

Throughout this study, data are presented as means \pm S.E.M. The statistical significance of the difference between the groups was determined using SigmaStat (version 3.5, Systat Software, USA).

Materials

Tissue culture media were obtained from Invitrogen (France). BrdU was obtained from Serva (France). All other reagents were from Sigma-Aldrich (France). Hyperforin was a kind gift from Dr Willmar Schwabe GmbH & Co. (Germany). The extract was a mixture of hyperforin with its homologue adhyperforin (ratio 8:2), prepared as sodium salts (Tu *et al.* 2009).

Results

The consequences of a chronic hyperforin treatment on cell proliferation, cell survival and adult hippocampal neurogenesis were analysed in adult mice after 4 wk of daily i.p. injections (4 mg/kg) of hyperforin. The mice then received two injections of BrdU to label dividing cells followed by a continuous treatment of hyperforin for 3 more weeks. The chronic hyperforin treatment had no effect on cell proliferation as measured by the number of phosphorylated histone H3 by means of an anti-phospho-histone H3 that recognizes histone H3 when phosphorylated at Ser¹⁰ (pH 3, Fig. 1a). The survival of 3-wk-old BrdU-labelled cells was also not modified (Fig. 1b). As a consequence, neurogenesis, evaluated by the number of DCX, remained unchanged (Fig. 1c). Thus, under the experimental conditions used, hyperforin did not influence hippocampal adult neurogenesis.

The effects of hyperforin on expression of TrkB in the cortex and hippocampus were also addressed. Adult mice were chronically treated for 4 wk (4 mg/kg.d) with hyperforin. This treatment enhanced the expression of TrkB and p-TrkB in the cortex by nearly 124% and 200% ($p < 0.05$), respectively (Fig. 2a,b).

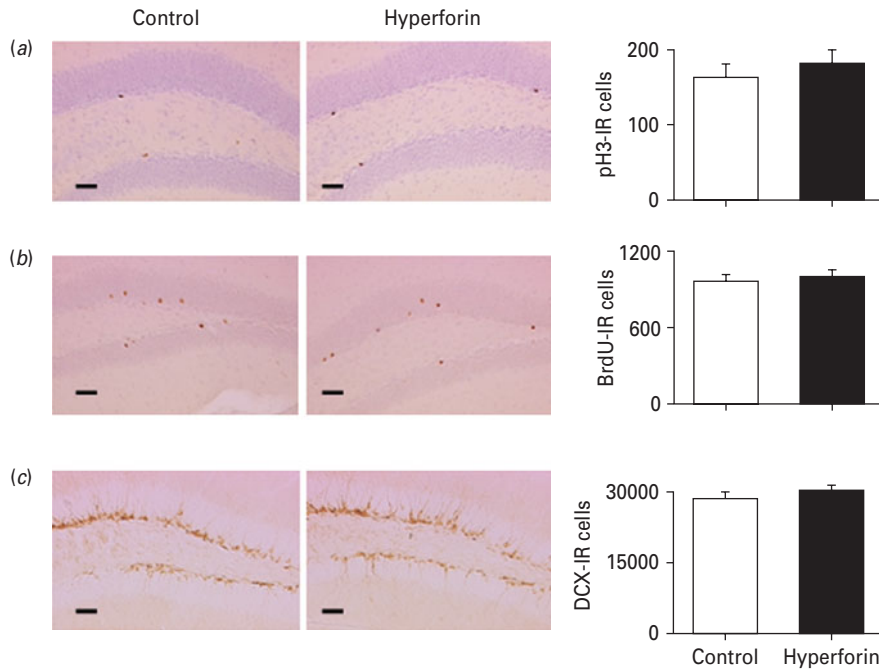


Fig. 1. Hyperforin does not influence adult hippocampal neurogenesis. Ten control (left panel) and ten treated animals (middle panel) received, respectively, a daily injection of 0.9% NaCl or 4 mg/kg hyperforin for 4 wk. This treatment was followed by two injections of 5-bromo-2'-deoxyuridine (BrdU) (at 24-h intervals) and animals were treated for 3 more weeks with 4 mg/kg hyperforin (or with 0.9% NaCl). Animals were killed and brain slices were immunostained with antibodies against pH3, BrdU and doublecortin (DCX). Representative photomicrographs with immunostained cells localized in the dentate gyrus are shown (left and middle panels). The total number of pH3-immunoreactive (IR) (a), BrdU-IR (b) and DCX-IR (c) cells is also given (right panels). No significant differences were observed between groups for cell proliferation (a), cell survival (b) and neurogenesis (c). Scale bar, 50 μ m.

The expression of CREB and p-CREB, although slightly augmented, was nevertheless not different when compared to their respective controls (NaCl-treated animals) ($p > 0.05$). In marked contrast to the cortex, the expression of the above-mentioned proteins in the hippocampus was not modulated by hyperforin (Fig. 2a,b). This indicates that hyperforin exerts tissue-specific responses.

Since hyperforin influences the expression of TrkB in the cortex but not in the hippocampus, cultures of cortical neurons were prepared to uncover the mechanisms by which this plant extract alters the BDNF pathway. We first determined whether a chronic application of hyperforin changes the expression of CREB, p-CREB, TrkB and p-TrkB (Fig. 3a). A 72 h hyperforin treatment enhanced the expression of CREB, p-CREB and TrkB in cultured cortical neurons by 70%, 109% and 71%, respectively ($p < 0.05$) (Fig. 1b). However, p-TrkB remained unaffected.

It was recently shown that a chronic hyperforin treatment in adult mice influences Zn^{2+} storage capacities of brain cells by stimulating the expression of

metallothioneins (Gibon *et al.* 2011). Since Zn^{2+} interferes with the activity of the BDNF/TrkB pathway by activating TrkB independent of its ligand (Huang *et al.* 2008), we investigated whether the Zn^{2+} chelator TPEN could prevent the hyperforin-dependent regulation of p-CREB and TrkB. Cortical neurons in culture were kept for 72 h with a medium supplemented with either 1 μ M TPEN or 1 μ M TPEN + 1 μ M hyperforin. With TPEN, hyperforin was still able to enhance the phosphorylation of CREB (Fig. 3c) showing that Zn^{2+} does not play a role in regulating the hyperforin-dependent cascade leading to increases in the expression of CREB and its phosphorylated form. However, in the presence of TPEN, hyperforin failed to up-regulate TrkB expression (Fig. 3d) suggesting that this metal controls a step in between the transcription factor p-CREB and the expression of TrkB.

Since hyperforin selectively enhances the activity of the Ca^{2+} -conducting TRPC6 channels, while leaving the other TRPC channels unaffected (Leuner *et al.* 2007), it is postulated that hyperforin could influence neuronal properties by regulating the cytosolic

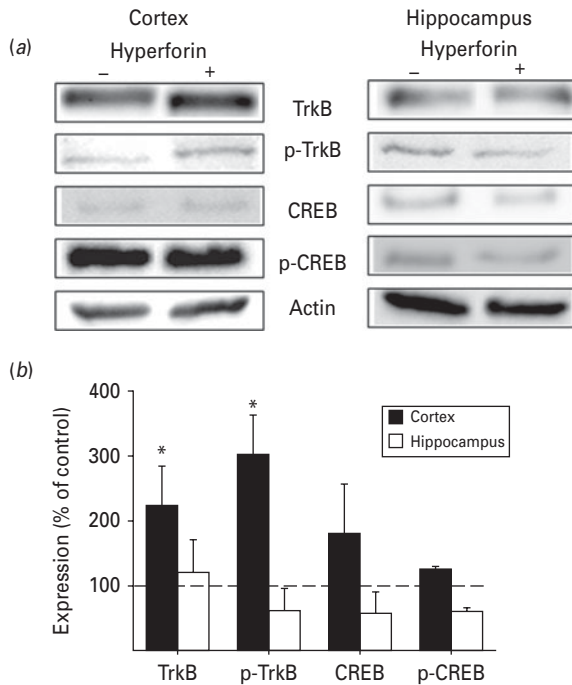


Fig. 2. Hyperforin regulates the expression of TrkB in the cortex but not in the hippocampus of adult mice. (a) Representative Western blots showing the expression of TrkB, p-TrkB, CREB and p-CREB in control animals (NaCl, $n=3$) and in animals treated for 4 wk with hyperforin (i.p., 4 mg/kg.d, $n=3$). (b) The panel shows the changes in the expression level of TrkB, p-TrkB, CREB and p-CREB in both groups in the cortex and (black bar) and the hippocampus (white bar). * $p < 0.05$ (Kruskal–Wallis one-way analysis of variance followed by a Holm–Sidak test).

concentration of Ca^{2+} . Experiments were performed to verify whether Ca^{2+} channels were implicated in the hyperforin-dependent regulation of TrkB expression. Cortical neurons were kept in a medium containing the Ca^{2+} channel blocker SKF-96365 ($5 \mu\text{M}$) alone or SKF-96365 ($5 \mu\text{M}$) + hyperforin ($1 \mu\text{M}$). In the latter case, SKF-96365 was added 10 min before the addition of hyperforin. In the presence of SKF-96365, hyperforin did not enhance the phosphorylation of CREB (Fig. 3c). It also failed to up-regulate the expression of TrkB (Fig. 3d). The Ca^{2+} chelator BAPTA-AM was used to determine whether Ca^{2+} controls the phosphorylation of CREB and TrkB expression. Cortical neurons were thus grown in a medium containing 100 nM BAPTA-AM. Preliminary experiments showed that depending on the concentration used (e.g. at $\geq 1 \mu\text{M}$), a sustained application of BAPTA-AM resulted in cell loss (not shown). Interestingly, low concentrations of BAPTA-AM were sufficient to disturb the hyperforin-dependent expression of p-CREB

and TrkB (Fig. 3c,d). These results obtained with SKF-96365 and BAPTA suggest that Ca^{2+} is a central component, playing a role downstream of hyperforin and upstream of p-CREB and TrkB. Furthermore, cAMP-dependent PKA is known to control CREB activity (Kingsbury *et al.* 2003; Lonze & Ginty, 2002). Thus, its involvement in the hyperforin-dependent phosphorylation of CREB was investigated. Cells were incubated with hyperforin ($1 \mu\text{M}$) or with hyperforin + RpcAMP ($20 \mu\text{M}$), a competitive inhibitor of PKA (Murray, 2008). Under the latter condition, the expression of TrkB and p-CREB was not up-regulated (Fig. 3c,d). Instead, the expression of TrkB and p-CREB was reduced by 9% and 34%, respectively. The data summarized in Fig. 3c,d suggest that SKF-96365-sensitive channels, Ca^{2+} ions and PKA are potentially crucial candidates participating in the hyperforin-dependent regulation of TrkB expression.

The results obtained from the SKF-96365 and BAPTA experiments suggest that hyperforin acts on Ca^{2+} -conducting channels. TRPC6 channels are the sole Ca^{2+} channels activated by hyperforin (Leuner *et al.* 2007). It is important to note that the expression of hippocampal TRPC6 channels increases from postnatal day 0 to postnatal day 14 and then strongly declines, with a weak expression observed during adulthood (Tai *et al.* 2008). This developmental regulation in the expression pattern of hippocampal TRPC6 channels could explain, at least partially, the lack of responsiveness of this brain structure to hyperforin. The expression of TRPC6 channels in the adult mouse brain was investigated by Western blotting (Fig. 4a). In the cortex of control animals, the level of TRPC6 expression was around four times higher than that observed in the hippocampus (Fig. 4b). In addition, hyperforin treatment increased the expression of TRPC6 in the cortex ($p < 0.05$) (Fig. 4b) but not in the hippocampus ($p > 0.05$) (Mann–Whitney) (Fig. 4b).

Discussion

SJW is one of the most commonly used medicinal plant (Wills *et al.* 2000). Since the first meta-analysis of Linde *et al.* (1996) showing that extracts of SJW were as effective as standard antidepressants for the treatment of some forms of mood disorders (Linde *et al.* 1996), the usefulness of this medicinal plant has been documented (Di Carlo *et al.* 2001; Stevinson & Ernst, 1999). Although several constituents of SJW seem to contribute to its antidepressive action, the phloroglucinol derivative hyperforin is considered as its main effector (Cervo *et al.* 2002; Chatterjee *et al.* 1998a). Due to its recognized clinical efficacy, SJW is regarded as an

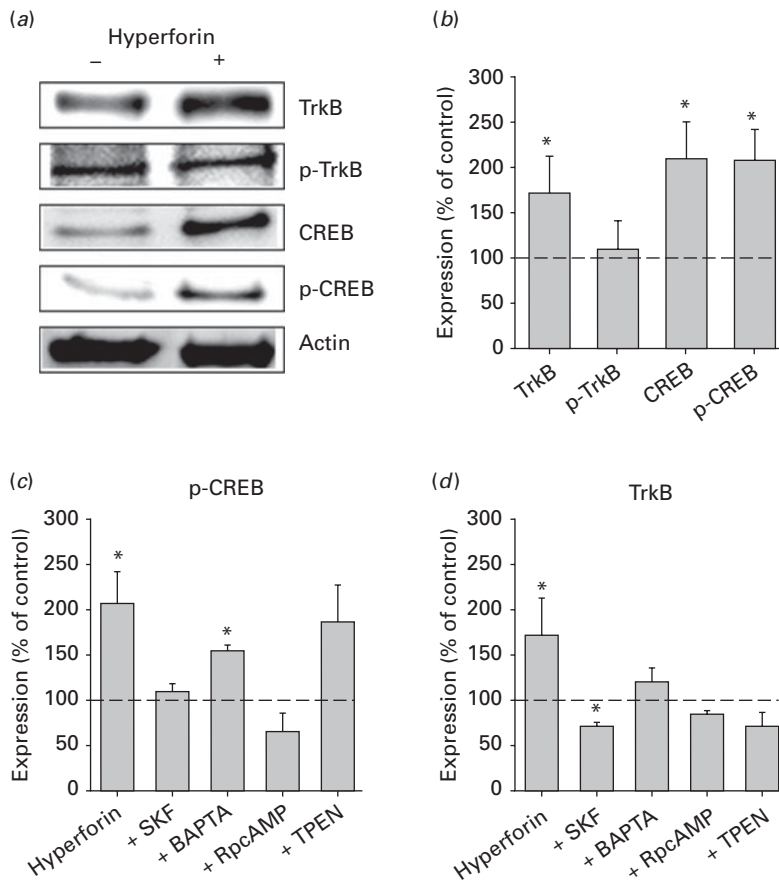


Fig. 3. Hyperforin enhances the expression of TrkB, CREB and p-CREB in cultured cortical neurons. Western blot analysis of the expression of TrkB, p-TrkB, CREB and p-CREB in cortical neurons kept in culture for 72 h in the presence of 1 μ M hyperforin. (a) Representative Western blots from control neurons (–) and neurons treated with hyperforin (+). (b) Quantitative presentation of the expression of each protein. Expression levels were normalized to the expression level of actin and are represented in % of the expression of each protein under control conditions. Data are collected from at least three independent experiments. * $p < 0.05$ (Kruskal–Wallis followed by a Holm–Sidak test). (c) and (d) Western blot analysis of the expression of p-CREB (c) and TrkB (d) in cortical neurons kept in culture for 72 h in the presence of 1 μ M hyperforin without or with the channel blocker SKF-96365 (5 μ M), the Ca^{2+} chelator BAPTA/AM (100 nM), or the PKA inhibitor RpcAMP (20 μ M). * $p < 0.05$ (Kruskal–Wallis followed by a Holm–Sidak test). In some experiments, cortical neurons in culture were kept for 72 h with a medium supplemented with either 1 μ M TPEN or 1 μ M TPEN + 1 μ M hyperforin.

alternative treatment for depression. However, the mechanisms by which it acts on neuronal functions and those underlying its behavioural responses are still obscure.

In-vitro and *in-vivo* experiments illustrate the positive influence of a chronic hyperforin treatment on the expression of TrkB. This up-regulation is observed on cultured cortical neurons from embryonic mice (*in vitro* data) as well as on brain from adult mice (*in-vivo* data). The hyperforin-dependent regulation of the gene coding for this BDNF receptor can be observed in cultured neurons from immature brains and *in vivo* on mature brains. The hyperforin-dependent expression of TrkB is abolished in the presence of the

Ca^{2+} channel blocker SKF-96365, is sensitive to the Ca^{2+} chelator BAPTA, critically depends on the activity of the serine-threonine PKA and involves the inducible transcription factor CREB. Upon binding to gene promoters p-CREB, the active form of CREB, controls the expression of various proteins like TrkB (Lonze & Ginty, 2002). The action of this transcription factor involves a transcriptional co-activator, the CREB-binding protein (CBP). It is a Zn^{2+} -binding protein participating in the regulation of transcription (Legge *et al.* 2004; Newton *et al.* 2000). TPEN may block the expression of TrkB by altering the functions and/or the folding of this Zn^{2+} -binding transcriptional co-activator. Figure 5 is a schematic representation

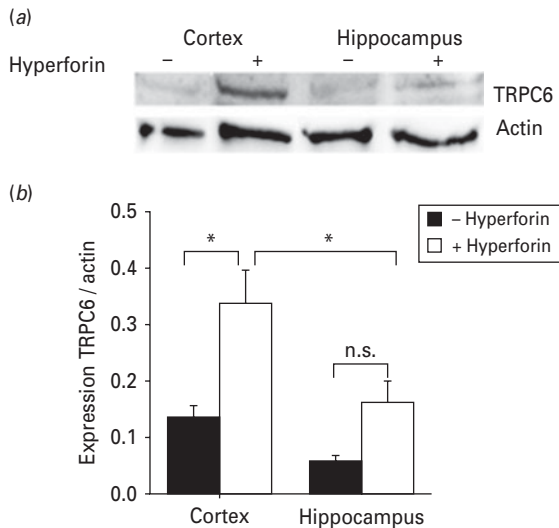


Fig. 4. TRPC6 expression in the cortex and hippocampus of mice adult brain. (a) Representative Western blots showing the expression of TRPC6 and actin in the cortex and hippocampus of control (–) and hyperforin-treated animals (+). (b) Summary plot showing the expression of TRPC6 in control ($n=3$) (black bars) and in hyperforin-treated animals ($n=3$) (white bars). TRPC6 expression is expressed with respect to actin expression. * $p < 0.05$ (Mann–Whitney). Duration of treatment: 4 wk.

showing this hyperforin-dependent signalling pathway. Some differences can be noted when comparing the *in-vivo* and the *in-vitro* data. For instance, hyperforin strongly augments the expression of p-TrkB *in vivo* whereas no change was observed *in vitro*. These differences could be due to the different duration of the treatment (4 wk *in vivo* vs. 72 h *in vitro*) and to the concentrations of hyperforin used. But in both models (adult mice chronically treated and cortical neurons in cultures), the plant extract potentially enhances the expression of TrkB.

The findings of the present report show that hyperforin controls a SKF-96365-sensitive signalling cascade, involving PKA and CREB. TRPC6 being the only plasma membrane channels known to date to be activated by this agent, it is proposed that they mediate the hyperforin response. Ca^{2+} ions entering via hyperforin-sensitive TRPC6 channels seem to control, in a PKA-dependent manner, the phosphorylation of CREB and the downstream expression of TrkB. The mechanism by which Ca^{2+} ions seem to activate PKA in cortical neurons has not been addressed in the present study. Ca^{2+} ions, via their binding to calmodulin CaM, are able to activate PKA, making CREB a Ca^{2+} -inducible transcription factor (Lonze & Ginty,

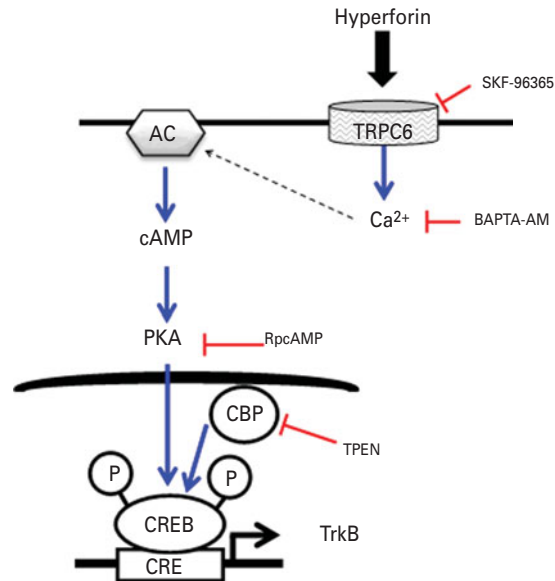


Fig. 5. Schematic representation showing the hyperforin-dependent signalling pathway in the cortex. Hyperforin activates SKF-96365-sensitive Ca^{2+} channels of the plasma membrane leading to cytosolic Ca^{2+} changes that can be prevented by the Ca^{2+} chelator BAPTA-AM. The hyperforin-triggered Ca^{2+} influx results in the activation of PKA which phosphorylates CREB, giving rise to enhanced levels of p-CREB. CBP mediates the interaction between the transcription factor CREB and the DNA. CBP is a transcriptional co-activator that possesses three regions that bind Zn^{2+} ions. AC, Adenylate cyclase; CBP, CREB-binding protein; PKA, protein kinase A.

2002). Importantly, the overexpression of TRPC6 augments the phosphorylation of CREB (Tai *et al.* 2008). We propose that Ca^{2+} entering through TRPC6 channels, indirectly activate PKA.

In the brain of adult mice, TRPC6 channels are present in the cortex but are, however, found at a much weaker level in the hippocampus where their expression is developmentally regulated (Tai *et al.* 2008). Indeed, TRPC6 expression increases from P0 to P14 and then strongly decreases (Tai *et al.* 2008). Of note, in the hippocampus of young mice, TRPC6 controls the neuritic outgrowth of immature hippocampal neurons and the formation of excitatory synapses (Tai *et al.* 2008; Zhou *et al.* 2008). Therefore, the entry of Ca^{2+} through TRPC6 is able to control downstream cellular responses. Our data support this view and are perfectly in line with reports showing that hyperforin is an activator of TRPC6 channels (Leuner *et al.* 2007; Muller *et al.* 2008). In addition, a chronic hyperforin treatment enhanced their expression further illustrating that TRPC6 is a target of this plant extract.

Although, a great number of studies have contributed to a better understanding of the molecular biology of depressive disorder, little is known regarding the precise mechanism of action of antidepressant drugs. According to some authors, the neurotrophin BDNF and the action of antidepressants on the hippocampal adult neurogenesis are two central features involving TrkB receptors. Indeed, some experimental data support the existence of a functional link between BDNF, TrkB, the adult hippocampal neurogenesis and the behavioural effects of antidepressant drugs (Li *et al.* 2008). According to this model, TrkB has a central role in the pathophysiology of depressive disorders. Of interest, hyperforin augments its expression. However, there is no unequivocal demonstration showing that the adult hippocampal neurogenesis is really necessary to produce the behavioural effects of antidepressants (Sahay *et al.* 2011). Although the BDNF-neurogenesis model has been well documented, it is debated (DeCarolis & Eisch, 2010; Sapolsky, 2004), notably because it seems to suffer some exceptions (Bessa *et al.* 2009; David *et al.* 2009; Surget *et al.* 2008) suggesting that it may not fully cover the neurobiology of all the antidepressants. First, a decrease in hippocampal neurogenesis does not induce a depressive-like behaviour, indicating that this process is not involved in the pathogenesis of depression (Revest *et al.* 2009; Santarelli *et al.* 2003). Importantly, the effects of antidepressants on neurogenesis seem to depend on the mouse strain used (Holick *et al.* 2008). In addition, antidepressants like fluoxetine seem to stimulate the hippocampal neurogenesis of mice under chronic stress but not when tested on unstressed animals (Surget *et al.* 2010). Another important parameter to consider when trying to understand the requirement (or not) of hippocampal neurogenesis in the efficacy of antidepressant drugs, is the type of antidepressant used. For instance, hippocampal neurogenesis underlines the action of monoaminergic compounds but does not seem to be required for drugs acting on the hypothalamo-pituitary-adrenal (HPA) axis (Surget *et al.* 2008).

In conclusion, *in-vitro* and *in-vivo* experiments were conducted to better address the CNS effects of the plant extract hyperforin. The data presented show that it controls a signalling cascade involving Ca^{2+} -conducting channels, PKA and CREB, which, in turn, up-regulates the expression of the BDNF receptor TrkB. Additionally, these responses are observed in the cortex but not in the hippocampus of adult mice where the expression of TRPC6 channels is weak. Altogether, the cortex appears as an important brain structure participating in the CNS effects of hyperforin.

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Statement of Interest

None.

References

- Albert D, Zundorf I, Dingermann T, Muller WE, *et al.* (2002). Hyperforin is a dual inhibitor of cyclooxygenase-1 and 5-lipoxygenase. *Biochemical Pharmacology* **64**, 1767–1775.
- Beerhues L (2006). Hyperforin. *Phytochemistry* **67**, 2201–2207.
- Bessa JM, Ferreira D, Melo I, Marques F, *et al.* (2009). The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. *Molecular Psychiatry* **14**, 764–773, 739.
- Bouron A, Altafaj X, Boisseau S, De Waard M (2005). A store-operated Ca^{2+} influx activated in response to the depletion of thapsigargin-sensitive Ca^{2+} stores is developmentally regulated in embryonic cortical neurons from mice. *Brain Research Developmental Brain Research* **159**, 64–71.
- Cervo L, Rozio M, Ekalle-Soppo CB, Guiso G, *et al.* (2002). Role of hyperforin in the antidepressant-like activity of Hypericum perforatum extracts. *Psychopharmacology (Berlin)* **164**, 423–428.
- Chatterjee S, Filippov V, Lishko P, Maximyuk O, *et al.* (1999). Hyperforin attenuates various ionic conductance mechanisms in the isolated hippocampal neurons of rat. *Life Sciences* **65**, 2395–2405.
- Chatterjee SS, Bhattacharya SK, Wonnemann M, Singer A, *et al.* (1998a). Hyperforin as a possible antidepressant component of hypericum extracts. *Life Sciences* **63**, 499–510.
- Chatterjee SS, Noldner M, Koch E, Erdelmeier C (1998b). Antidepressant activity of hypericum perforatum and hyperforin: the neglected possibility. *Pharmacopsychiatry* **31** (Suppl. 1), 7–15.
- Chen Y, Goldstein JA (2009). The transcriptional regulation of the human CYP2C genes. *Current Drug Metabolism* **10**, 567–578.
- David DJ, Samuels BA, Rainer Q, Wang JW, *et al.* (2009). Neurogenesis-dependent and -independent effects of

- fluoxetine in an animal model of anxiety/depression. *Neuron* **62**, 479–493.
- DeCarolis NA, Eisch AJ** (2010). Hippocampal neurogenesis as a target for the treatment of mental illness: a critical evaluation. *Neuropharmacology* **58**, 884–893.
- Di Carlo G, Borrelli F, Ernst E, Izzo AA** (2001). St John's wort: Prozac from the plant kingdom. *Trends in Pharmacological Sciences* **22**, 292–297.
- Duman RS, Monteggia LM** (2006). A neurotrophic model for stress-related mood disorders. *Biological Psychiatry* **59**, 1116–1127.
- Dupret D, Fabre A, Dobrossy MD, Panatier A, et al.** (2007). Spatial learning depends on both the addition and removal of new hippocampal neurons. *PLoS Biology* **5**, e214.
- Dupret D, Revest JM, Koehl M, Ichas F, et al.** (2008). Spatial relational memory requires hippocampal adult neurogenesis. *PLoS ONE* **3**, e1959.
- Fisunov A, Lozovaya N, Tsintsadze T, Chatterjee S, et al.** (2000). Hyperforin modulates gating of P-type Ca^{2+} current in cerebellar Purkinje neurons. *Pflugers Archiv: European Journal of Physiology* **440**, 427–434.
- Gey C, Kyrilenko S, Hennig L, Nguyen LH, et al.** (2007). Phloroglucinol derivatives guttiferone G, aristoforin, and hyperforin: inhibitors of human sirtuins SIRT1 and SIRT2. *Angewandte Chemie International Edition* **46**, 5219–5222.
- Gibon J, Richaud P, Bouron A** (2011). Hyperforin changes the zinc-storage capacities of brain cells. *Neuropharmacology* **61**, 1321–1326.
- Holick KA, Lee DC, Hen R, Dulawa SC** (2008). Behavioral effects of chronic fluoxetine in BALB/cJ mice do not require adult hippocampal neurogenesis or the serotonin 1A receptor. *Neuropsychopharmacology* **33**, 406–417.
- Huang YZ, Pan E, Xiong ZQ, McNamara JO** (2008). Zinc-mediated transactivation of TrkB potentiates the hippocampal mossy fiber-CA3 pyramid synapse. *Neuron* **57**, 546–558.
- Kingsbury TJ, Murray PD, Bambrick LL, Krueger BK** (2003). Ca^{2+} -dependent regulation of TrkB expression in neurons. *Journal of Biological Chemistry* **278**, 40744–40748.
- Koeberle A, Rossi A, Bauer J, Dehm F, et al.** (2011). Hyperforin, an anti-inflammatory constituent from St. John's wort, inhibits microsomal prostaglandin E(2) synthase-1 and suppresses prostaglandin E(2) formation *in vivo*. *Frontiers in Pharmacology* **2**, 7.
- Kozisek ME, Middlemas D, Bylund DB** (2008). Brain-derived neurotrophic factor and its receptor tropomyosin-related kinase B in the mechanism of action of antidepressant therapies. *Pharmacology and Therapeutics* **117**, 30–51.
- Kumar V, Mdzinarishvili A, Kiewert C, Abbruscato T, et al.** (2006). NMDA receptor-antagonistic properties of hyperforin, a constituent of St. John's wort. *Journal of Pharmacological Sciences* **102**, 47–54.
- Legge GB, Martinez-Yamout MA, Hambly DM, Trinh T, et al.** (2004). ZZ domain of CBP: an unusual zinc finger fold in a protein interaction module. *Journal of Molecular Biology* **343**, 1081–1093.
- Leuner K, Heiser JH, Derksen S, Mladenov MI, et al.** (2010). Simple 2,4-diacylphloroglucinols as classic transient receptor potential-6 activators – identification of a novel pharmacophore. *Molecular Pharmacology* **77**, 368–377.
- Leuner K, Kazanski V, Muller M, Essin K, et al.** (2007). Hyperforin – a key constituent of St. John's wort specifically activates TRPC6 channels. *FASEB Journal* **21**, 4101–4111.
- Li Y, Luikart BW, Birnbaum S, Chen J, et al.** (2008). TrkB regulates hippocampal neurogenesis and governs sensitivity to antidepressive treatment. *Neuron* **59**, 399–412.
- Linde K, Ramirez G, Mulrow CD, Pauls A, et al.** (1996). St John's wort for depression – an overview and meta-analysis of randomised clinical trials. *British Medical Journal* **313**, 253–258.
- Lonze BE, Ginty DD** (2002). Function and regulation of CREB family transcription factors in the nervous system. *Neuron* **35**, 605–623.
- Lorusso G, Vannini N, Sogno I, Generoso L, et al.** (2009). Mechanisms of Hyperforin as an anti-angiogenic angioprevention agent. *European Journal of Cancer* **45**, 1474–1484.
- Medina MA, Martinez-Poveda B, Amores-Sanchez MI, Quesada AR** (2006). Hyperforin: more than an antidepressant bioactive compound? *Life Sciences* **79**, 105–111.
- Mennini T, Gobbi M** (2004). The antidepressant mechanism of *Hypericum perforatum*. *Life Sciences* **75**, 1021–1027.
- Miguel-Hidalgo JJ, Rajkowska G** (2002). Morphological brain changes in depression: can antidepressants reverse them? *CNS Drugs* **16**, 361–372.
- Moore LB, Goodwin B, Jones SA, Wisely GB, et al.** (2000). St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. *Proceedings of National Academy of Sciences USA* **97**, 7500–7502.
- Muller M, Essin K, Hill K, Beschmann H, et al.** (2008). Specific TRPC6 channel activation, a novel approach to stimulate keratinocyte differentiation. *Journal of Biological Chemistry* **283**, 33942–33954.
- Muller WE** (2003). Current St John's wort research from mode of action to clinical efficacy. *Pharmacological Research* **47**, 101–109.
- Murray AJ** (2008). Pharmacological PKA inhibition: all may not be what it seems. *Sciences Signaling* **1**, re4.
- Newton AL, Sharpe BK, Kwan A, Mackay JP, et al.** (2000). The transactivation domain within cysteine/histidine-rich region 1 of CBP comprises two novel zinc-binding modules. *Journal of Biological Chemistry* **275**, 15128–15134.
- Revest JM, Dupret D, Koehl M, Funk-Reiter C, et al.** (2009). Adult hippocampal neurogenesis is involved in anxiety-related behaviors. *Molecular Psychiatry* **14**, 959–967.
- Roz N, Rehavi M** (2004). Hyperforin depletes synaptic vesicles content and induces compartmental redistribution of nerve ending monoamines. *Life Sciences* **75**, 2841–2850.
- Saddique Z, Naeem I, Maimoona A** (2010). A review of the antibacterial activity of *Hypericum perforatum* L. *Journal of Ethnopharmacology* **131**, 511–521.

- Sahay A, Hen R** (2007). Adult hippocampal neurogenesis in depression. *Nature Neurosciences* **10**, 1110–1115.
- Sahay A, Scobie KN, Hill AS, O'Carroll CM, et al.** (2011). Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature* **472**, 466–470.
- Santarelli L, Saxe M, Gross C, Surget A, et al.** (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**, 805–809.
- Sapolsky RM** (2004). Is impaired neurogenesis relevant to the affective symptoms of depression? *Biological Psychiatry* **56**, 137–139.
- Schempp CM, Pelz K, Wittmer A, Schopf E, et al.** (1999). Antibacterial activity of hyperforin from St John's wort, against multiresistant *Staphylococcus aureus* and Gram-positive bacteria. *Lancet* **353**, 2129.
- Stevinson C, Ernst E** (1999). Hypericum for depression. An update of the clinical evidence. *European Neuropsychopharmacology* **9**, 501–505.
- Surget A, Saxe M, Leman S, Ibarguen-Vargas Y, et al.** (2008). Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. *Biological Psychiatry* **64**, 293–301.
- Surget A, Tanti A, Leonardo ED, Laugeray A, et al.** (2010). Antidepressants recruit new neurons to improve stress response regulation. *Molecular Psychiatry* **16**, 1177–1188.
- Tai Y, Feng S, Ge R, Du W, et al.** (2008). TRPC6 channels promote dendritic growth via the CaMKIV-CREB pathway. *Journal of Cell Sciences* **121**, 2301–2307.
- Tu P, Kunert-Keil C, Lucke S, Brinkmeier H, et al.** (2009). Diacylglycerol analogues activate second messenger-operated calcium channels exhibiting TRPC-like properties in cortical neurons. *Journal of Neurochemistry* **108**, 126–138.
- Wills RB, Bone K, Morgan M** (2000). Herbal products: active constituents, modes of action and quality control. *Nutrition Research Reviews* **13**, 47–77.
- Zanoli P** (2004). Role of hyperforin in the pharmacological activities of St. John's wort. *CNS Drug Reviews* **10**, 203–218.
- Zhou J, Du W, Zhou K, Tai Y, et al.** (2008). Critical role of TRPC6 channels in the formation of excitatory synapses. *Nature Neurosciences* **11**, 741–743.