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Hypericum perforatum differentially affects corticosteroid receptor-mRNA expression in human monocytic U-937 cells

F. Enning, H. Murck, J.-C. Krieg, H. Vedder*

Department of Psychiatry and Psychotherapy, Philipps-University of Marburg, Rudolf-Bultmann-Str. 8, D-35033 Marburg, Germany

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ABSTRACT

A dysregulation of the hypothalamic-pituitary-adrenocortical (HPA) axis represents a prominent finding in major depression, possibly related to a dysfunction of the corticosteroid receptor system. Antidepressants are involved in the restoration of the altered feed-back mechanism of the HPA-axis, probably via normalization of corticosteroid receptor functions. Since Hypericum perforatum has antidepressive properties, we here examined its putative actions on glucocorticosteroid receptor mRNA levels in human blood cells as a peripheral model for neuroendocrine effects in human brain cells.

Our data show that Hypericum (LI 160) affects the cellular mRNA levels of both, the glucocorticoid receptor (GR)-α and its inhibitory counterpart, the GR-β, at clinically-relevant concentrations. Under these conditions, a bimodal effect was observed. Dose-response studies suggest a rather small effective concentration range and time-effect data show a primary and transient up-regulation of GR-α mRNA levels and a down-regulation of GR-β mRNA levels after 16 h of treatment. The sodium channel blocker benzamil neutralized the effects of Hypericum, pointing to an at least partial mechanism of action via this pathway.

In conclusion, Hypericum treatment differentially affects GR-mRNA levels in the human system. Our data suggest a bimodal effect on GR, resulting in a time-and dose-related modification of GR-mediated cellular effects. Such a mechanism has been alleged as an important way of action for a number of antidepressants. It is the first time that a specific effect on both receptors, especially on the subtype of GR-β, is shown under antidepressive treatment in a human system under in vitro conditions.

1. Introduction

A dysregulation of the hypothalamic-pituitary-adrenocortical (HPA) system represents one of the most prominent neurobiological alterations in major depressive disorder (MDD) (for review see: Holsboer, 1999; Gold and Chrousos, 2002). Recent studies reinforced the hypothesis, that glucocorticoid receptors are crucially involved in this dysfunction of the HPA-system (Holsboer, 2000; Pariante and Miller, 2001; Gold and Chrousos, 2002; Buckley et al., 2007; Claes, 2009; Kronenberg et al., 2009). These observations suggest, that the influence on neuroendocrine feed-back functions due to an action on GR functions and GR-mediated cellular effects represents one of the important mechanisms of the action of antidepressants (Holsboer and Barden, 1996; Gold and Chrousos, 2002; Chrousos and Kino, 2009). It was concluded, that this mechanism finally causes a normalization of HPA-regulated hormone secretion with subsequent clinical improvement in psychopathology (Reul et al., 1994; Barden et al., 1995; Holsboer and Barden, 1996).

This mode of action might also be one of the clues for the antidepressive effects of Hypericum perforatum (St. John's wort), the active antidepressive principles of which have not yet been finally elucidated. In spite of heterogeneous results of different studies regarding the antidepressive efficacy of St. John's wort, Hypericum (H.) is widely used as a treatment option for major depression in Europe and the United States (Shelton, 2009). Several reviews of controlled clinical studies demonstrate that Hypericum represents an effective antidepressant treatment superior to placebo (Linde et al., 1996; Volz, 1997; Wheatly, 1998; Wong et al., 1998), even if meta-analyses show varying results comparing the effectiveness of St. Johns' wort with other antidepressants. This might be caused by different study designs and probably also different preparations of Hypericum employed (Linde, 2008). A recent meta-analysis demonstrated an effectiveness equal to SSRI, although with less adverse events (Rahimi et al., 2009). Additionally, recent studies show that H. perforatum might be also primarily effective in depression with atypical symptoms (Murck et al., 2005). Neurobiologically, it is active in a large number of biochemical and

* Corresponding author. Present address: Psychiatric Centre Nordbaden, Wiesloch, Heidelberger Str. 1A, 69168 Wiesloch, Germany. Tel.: +49 6222 55 2000; fax: +49 6222 55 2912001.
E-mail address: helmut.vedder@pzn-wiesloch.de (H. Vedder).

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behavioural models which are indicative of antidepressant activity (Butterweck et al., 1997; Müller et al., 1997; Bhattacharya et al., 1998; Chatterjee et al., 1998, 1999; Gambarana et al., 1999; Singewald et al., 2004).

Regarding effects on neurotransmission, it has been shown that Hypericum inhibits the neuronal uptake of serotonine, norepinephrine, dopamine, L-glutamate and GABA (Wonnemann et al., 2000). No other antidepressant exhibits a similar broad uptake-inhibiting profile. This effect is associated with increased free intracellular sodium concentrations (Singer et al., 1999). Especially the amiloride-sensitive sodium conductive pathways and Na^+- H^+-exchangers may play an important role in the modulation of the neuronal uptake of the different neurotransmitters (Wonnemann et al., 2000).

An important basic mechanism of the therapeutic effects of antidepressants might lie in the normalization of altered functions of the HPA-system, involved in the pathophysiology of depressive disorder, via direct effects on GRs (Alt et al., 2010). Thus, the basic hypothesis of the present study consists of the assumption that H. perforatum affects the GR system via modulation of GR-mRNA levels. This would add a further relevant mechanism of action to the broad antidepressive effect spectrum of Hypericum in MDD.

The effects of Hypericum on both types of GR receptors, GR-α and GR-β, were examined in the immunological model system of U-937 cells, a human monocytic cell line, which allows the study of effects in a relevant human system (Vedder et al., 1999; Heiske et al., 2003). Moreover, we wanted to examine whether the effect of Hypericum on GR-mRNA expression is based on the increase in intracellular sodium concentrations, already described as the mechanism of action for its serotonine reuptake inhibition properties (Singer et al., 1999). Therefore, we blocked the potentially responsible conductive pathways with several specific agents. We used the Na^+ ionophore monensin, the Na^+—K^+ pump inhibitor ouabain and the sodium-calcium exchange blocker and amilorid-analogon benzamil.

2. Methods

2.1. U-937 cells and culture conditions

U-937 cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% heat-inactivated foetal calf serum (FCS) (Gibco/BRL and Seromed, Berlin, Germany), 0.5% glutamine, and 1% gentamicine at 37 °C in a 5% CO_2 atmosphere. For the studies, cells were plated at a concentration of 225,000 cells in 3 ml medium per well into six-well culture plates (Greiner, Frickenhausen, Germany).

For the examination of dose-related effects, cells were preincubated for 24 h and then treated with Hypericum-extract, dissolved in DMSO (4%) at a maximal concentration of 100 μg Hypericum/ml, and at further concentrations of 50 μg/ml, 25 μg/ml, 12.5 μg/ml and 6.25 μg/ml, established by a dilution series. These were added to the plates at a quantity of 300 μl to each well. The same procedure was performed with the DMSO controls without Hypericum. The Hypericum extract LI 160 was a kind gift of Lichtwer Pharma GmbH, Berlin.

For the examination of time-related effects, cells were incubated for 4, 16, 24, 48 h with Hypericum extract, dissolved in DMSO (4%) at a concentration of 2.5 μg/ml. The same procedure was performed using DMSO as a control.

For further experiments on the underlying mechanisms of action (blockade of sodium conductive pathways), cells were pre-incubated for 24 h and then treated with Hypericum extract at a concentration of 2.5 μg/ml plus monensin at concentrations of 0.1 and 0.5 μM, ouabain at concentrations of 0.1 and 0.5 μM and benzamil at concentrations of 1.0 and 25.0 μM. Additionally, cells were incubated with monensin, ouabain and benzamil without Hypericum as respective controls.

2.2. RNA extraction

After incubation, the cells were collected from the culture dishes and total RNA was extracted by the use of Trizol® reagent in accordance with the manufacturer's instructions. The amount of extracted total RNA was quantified by established optical methods at A_{260}/A_{280} (Genequant II, Pharmacia Biotech, Freiburg, Germany) and structural integrity checked by agarose-gel electrophoresis (1.5% agarose (Gibco/BRL)).

2.3. Reverse transcriptase-polymerase chain reaction (RT-PCR)

RT-PCR was used to analyse the transcription of the GRs and the house-keeping genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-Actin. 1 μg of cellular total RNA was reverse-transcribed with 40 U of Superscript II (Gibco/BRL) and 1 μg oligo-(dT) in a volume of 20 μl following the manufacturer's protocol.

The following primer pairs were used to amplify the cDNA's:

GR-α	(5′ primer:	5′-;CCT AAG GAC GGT CTG AAG AGC-3′;
	3′ primer:	5′-GCC AAG TCT TGG CCC TCT AT-3′) (477 bp);
GR-β	(5′ primer:	5′-CCT AAG GAC GGT CTG AAG AGC-3′;
	3′ primer:	5′-CCA CGT ATC CTA AAA GGG CAC-3′) (367 bp);
GAPDH	(5′ primer:	5′-CGT CTT CAC CAC CAT GGA GA-3′
	3′ primer:	5′-CGG CCA TCA CGC CAC AGT TT-3′) (788 bp);
β-Actin	(5′ primer	5′-GAG GCC CAG AGC AAG AGA GG -3′
	3′ primer	5′-TCA CCG GAG TCC ATC ACG GAT -3′) (302 bp).

Aliquots of 1 μl cDNA were amplified with a PCR cycler (Biometra Trio, Göttingen, Germany) for GR and GAPDH using the primers described above with the following cycling program: denaturation for 45 s at 95 °C, annealing for 60 s at 59 °C, and extension for 60 s at 72 °C. PCR products were analysed for GR (28 cycles), GAPDH (28 cycles) and β-Actin (28 cycles) after amplification and gel electrophoresis in 1.5% agarose gels. Semi-quantitative determination was achieved by digitization of gels with a Polaroid video system (Rothaar & Schroeder, Heidelberg, Germany) and further densitometric evaluation achieved with the Gelscan 4.0 Professional Program (LTF/BioSciTec, Landau/Frankfurt, Germany).

The used primer sequences were derived from Oakley et al. (1996). They showed that the alpha and beta isoforms of the human glucocorticoid receptor were identical through amino acid 727 but diverged beyond this position. GR-α has additional 50 amino acids and GR-β has additional nonhomologous 15 amino acids. This leads to a different size of the expression products, which allows to identify

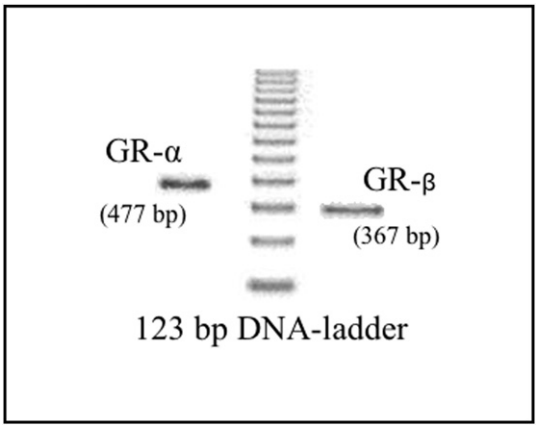

Fig. 1. RT-PCR of GR-α and GR-β mRNA expression in U-937 cells after treatment with 2.5 μg/ml of Hypericum perforatum. Note the different size of the PCR-products (GR-α = 477 bp and GR-β = 367 bp).