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Antidepressant-like effect of hyperfoliatin, a polyisoprenylated phloroglucinol derivative from *Hypericum perfoliatum* (Clusiaceae) is associated with an inhibition of neuronal monoamines uptake

Jean-Claude do Rego [a,*], Naïma Benkiki [b], Elizabeth Chosson [c], Zahia Kabouche [b], Elisabeth Seguin [c], Jean Costentin [a]

[a] *CNRS FRE 2735, Laboratory of Experimental Neuropsychopharmacology, European Institute for Peptide Research (IFRMP 23), Faculty of Medicine and Pharmacy, Institute of Biomedical Research, University of Rouen, 22 Boulevard Gambetta, 76183 Rouen Cedex, France*
[b] *Laboratoire d'Obtention de Substances Thérapeutiques (LOST), Faculty of Sciences, University of Mentouri - Constentine, Campus Chaabet Ersas, 25000 Constantine, Algeria*
[c] *CNRS UMR 6014, Laboratory of Pharmaceutical Chemistry, Faculty of Medicine and Pharmacy, University of Rouen, 22 Boulevard Gambetta, 76183 Rouen Cedex, France*

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Abstract

This study investigated, in mice, the antidepressant like effect of hyperfoliatin, a prenylated phloroglucinol derivative isolated from the aerial parts of *Hypericum perfoliatum*, as well as its action on monoaminergic systems. In the forced-swimming test, hyperfoliatin dose-dependently reduced immobility time. Immobility was interpreted as an expression of "behavioural despair", which could be a component of depression syndrome. The effect of hyperfoliatin did not result from the stimulation of animal motor activity. Hyperfoliatin inhibited, in a concentration-dependent manner, the [^{3}H]-dopamine, [^{3}H]-serotonin and [^{3}H]-noradrenaline synaptosomal uptakes, but did not prevent the binding of specific ligands to the monoamine transporters. These data suggest that the antidepressant-like effect of hyperfoliatin on the forced-swimming test is probably associated to monoamine uptake inhibition, due to a mechanism of action different from that of known antidepressants.

Keywords: Antidepressant drug; Monoaminergic system; Forced-swimming test; *Hypericum perfoliatum*; Hyperfoliatin

1. Introduction

The genus *Hypericum* L. (Clusiaceae) includes numerous species which have been used as medicinal plants for centuries in the treatment of trauma, burns, rheumatism, neuralgia, gastroenteritis, ulcers, hysteria, bedwetting and depression (Miller, 1998). In the past two decades several studies have demonstrated that extracts of *Hypericum* species contain antidepressant properties, as the tricyclic antidepressant, in humans (Ernst, 1995; Linde et al., 1996; Volz, 1997; Stevinson and Ernst, 1999) and exert an antidepressant-like action in laboratory animals (Bhattacharya et al., 1998; Butterweck et al., 1998; Müller et al., 1998; Panocka et al., 2000; Daudt et al., 2000; Sanchez-Mateo et al., 2002; Müller, 2003; Viana et al., 2005). However, neither the mechanisms of action nor the identity of the active constituents has yet been completely understood. Naphtodianthrone hypericin has been considered as the most active constituent of European St John's wort, *Hypericum perforatum* L. (Butterweck et al., 1997). In fact, some reports still provide evidence for the role of hypericin in antidepressant activity of *H. perforatum* (Butterweck et al., 1998) and the majority of the phytomedicines available are standardized on this naphthodianthrone. Nevertheless, currently most researchers consider that the antidepressant effects are due to a variety of constituents rather than a single one (Chatterjee et al., 1998a; Butterweck et al., 2000). Xanthones (Rocha et al., 1994), flavonoids (Butterweck et al., 2000) and in particular phloroglucinol derivatives (Chatterjee et al., 1998b; Müller et al., 2001; Viana et al., 2005) are also considered as pharmacologically relevant compounds. Among these extensively studied

* Corresponding author. Tel.: +33 2 35148602; fax: +33 2 35148603.
E-mail address: jean-claude.dorego@univ-rouen.fr (J.-C. do Rego).

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from both chemical and pharmacological points of view, their biological properties appear to be correlated with their prenylation, thus hyperforin (Bystrov et al., 1975; Beerhues, 2006) and adhyperforin (Maisenbacher and Kovar, 1992) have emerged as the constituents responsible for the antidepressant activity of *H. perforatum* (Chatterjee et al., 1998b; Barnes et al., 2001; Jensen et al., 2001; Müller et al., 2001; Roz et al., 2002). Recently, we reported the structure determination of hyperfoliatin, a novel prenylated phloroglucinol derivative, isolated from the aerial parts of *Hypericum perfoliatum* L. locally used in folk medicine for wound healing and in the treatment of various bacterial diseases (Benkiki et al., 2003). Hyperfoliatin was also extracted from aerial parts of *Hypericum scabrum*, an Usbekistan medicinal plant (Tanaka et al., 2004).

In this study we further investigated about the hyperfoliatin antidepressant-like effect and its mechanism of action. For this purpose, we tested the hyperfoliatin potential antidepressant effect on an experimental model of depression, the forced-swimming test, then we attempted to assess its effects on the uptake complex of monoaminergic systems.

2. Materials and methods

2.1. Behavioural experiments

2.1.1. Animals

Male Swiss albinos CD1 mice (IFFA-CREDO/Charles River, Saint-Germain sur L'Arbresle, France), weighing 25–30 g, were housed 20 in Makrolon cages (L: 40 cm, W: 25 cm, H: 18 cm), with free access to water and food (U.A.R., Villemoisson sur Orge, France). The animals were kept in a ventilated room, at a temperature of 22 ± 1 °C, under a 12-h light/12-h dark cycle (lights on between 7:00 a.m. and 7:00 p.m.).

All the experiments were carried out between 9:00 a.m. and 6:00 p.m., in testing rooms adjacent to the animal stabulation rooms. Animal manipulations were performed according to the European Communities Council Directive of 24 November 1986 (86:609:EEC) and conducted by authorized investigators. Each animal was used once, then immediately sacrificed.

2.1.2. Drug and solution

Hyperfoliatin was diluted to a concentration of 80 mg/kg in saline with 2% cremophor and 2% DMSO, and then diluted in saline immediately before treatment.

2.1.3. Forced-swimming test

The forced-swimming test was essentially similar to that described by Porsolt et al. (1977), but used an apparatus with a larger Plexiglas cylinder (14 cm in diameter, instead of 10 cm) similar to that employed by Semba and Takahashi (1988) and Do-Rego et al. (2002, 2005), since Sunal et al. (1994) have established that a cylinder with a higher diameter decreases the number of false positive responses. The apparatus consisted of two Plexiglas cylinders (20 cm height, 14 cm internal diameter), placed side-by-side in a Makrolon cage (L: 38, W: 24, H: 18 cm) and separated by an opaque screen. The Makrolon cage was filled with water at 22 ± 1 °C, to a height of 12 cm instead of 6 cm as suggested by Porsolt et al. (1977), since, according to Petit-Demouliere et al. (2005), the depth of water is an important parameter to be considered as mice should not sense a limit under the level of water. The behaviour of the mice would indeed be altered if their tails touch the bottom of the cylinder.

Fifteen minutes before the test the animals were isolated in small individual cages (L: 25, W: 9, H: 8 cm) at an ambient temperature (22 ± 1 °C). Thirty or 60 min after i.p. injection with vehicle (saline containing 0.05% cremophor + 0.05% DMSO) or graded doses of hyperfoliatin, four mice were tested simultaneously for a 6-min period. Total duration of immobility was measured during three consecutive periods of 2 min, each using an automated image analysis system (Videotrack MV 45). The method was approved by the Regional Ethical Committee for Animal Experimentation (Normandy; no. N/09-04-04-11).

2.1.4. Measurement of locomotor activity

Locomotor activity was measured in a Digiscan actometer (Omnitech Electronics Inc, Colombus, OH, U.S.A.), which monitored the horizontal and the vertical movements of the animals. The animals were placed individually in 20 × 20 × 30 cm compartments, placed into a dimly lit and quiet room. The recording apparatus was connected to a Compaq Computer in order to process the data. The responses to hyperfoliatin, injected intraperitoneally immediately before the test, were expressed as the number of crossed beams, during four consecutive 15 min periods.

2.2. In vitro assays

2.2.1. Animals

Male Sprague Dawley rats, weighting 180–200 g, were purchased from IFFA-CREDO/Charles River Laboratories (Domaine des Oncins, Saint-Germain sur L'Arbresle, France). They were housed by 4 in Makrolon cages (L: 40 cm, W: 25 cm, H: 18 cm), with free access to water and food (U.A.R., France) and kept in a well ventilated room, at a temperature of 21 ± 1 °C, under a 12-h light dark cycle (lights on between 7-h and 19-h). The procedures used in this study are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2.2. Drugs

[^{3}H]-dopamine (48 Ci/mmol) and [^{3}H]-nisoxetine (86 Ci/mmol) were purchased from Amersham (Les Ulis, France). [^{3}H]-noradrenaline (12.5 Ci/mmol), [^{3}H]-serotonin (25.5 Ci/mmol), [^{3}H]-citalopram (84.2 Ci/mmol) and [^{3}H]-mazindol (24.5 Ci/mmol) were purchased from Perkin Elmer-NEN Life Science Products (Paris, France). Cocaine hydrochloride was obtained from la Coopérative Pharmaceutique Française (Melun, France). Desipramine hydrochloride, fluoxetine hydrochloride and GBR 12783 were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Details of extraction, isolation and identification of hyperfoliatin have previously been reported (Benkiki et al., 2003). Solutions of hyperfoliatin (10^{-4}–10^{-10} M) were prepared in an incubation medium containing 0.05% cremophor and 0.05% DMSO. Millimolar solutions of GBR 12783 were prepared in